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Paul H. Furfey

THE FEMALE SEX HORMONE

THE FEMALE SEX HORMONE

PART I

BIOLOGY, PHARMACOLOGY AND CHEMISTRY

PART II

CLINICAL INVESTIGATIONS BASED ON THE
FEMALE SEX HORMONE BLOOD TEST

BY
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With 86 Illustrations and 36 Graphs

> *"Propter secretiones internas totas, mulier est quod est"*
> F. A. E. CREW [85]

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ACKNOWLEDGMENT

This Monograph, based upon twenty-five years of laboratory and clinical research, embodies and amplifies the subject as presented in two lectures delivered at the
University of Illinois School of Medicine
under the
Charles Sumner Bacon Lectureship
December 13 and 14, 1928

FOREWORD

THE writing of this monograph, long contemplated, was hastened and materialized by the invitation to deliver two lectures at the University of Illinois in inauguration of the Charles Sumner Bacon Lectureship in December of 1928. Dr. Charles S. Bacon, the well known gynecologist, emeritus professor of gynecology and obstetrics at the University of Illinois, in whose honor this lectureship was founded, personally requested that I present the results of my own investigations. In response to this wish, I collected and amplified my scattered writings and gave them the form of the present monograph.

My researches dealing with the female sex principle date back to the year 1904 and were continued more or less intermittently ever since, with the assistance and cooperation of numerous collaborators, among whom I must mention in particular the late Dr. Jacob Rosenbloom, the late Mr. T. Lefkowitz, Mr. O. I. Lee, Dr. P. M. Giesy, and at present, Prof. R. G. Gustavson, all occupied with the biochemical phases of the investigation. On the biological side of the research, I have been aided at some period of the work by Dr. Abe Unger of New York, Dr. H. N. Kingery, Mr. Claude D. Bonham of Denver, and by my present collaborators, Drs. Morris A. Goldberger and Gertrude Felshin of New York. A number of others have given me loyal help in various other phases of the work. For many years I have received much aid and encouragement from my wife, Marie-Louise Frank, whose suggestions and active cooperation have helped me in every detail of my investigations.

The first experiments, covering the period from 1904 to 1913, were performed in the Pathological Laboratory of the College of Physicians and Surgeons, Columbia University, of which the late Dr. Michael T. Prudden was director, with the exception of one year's work at the Rockefeller Institute, Director, Dr. Simon Flexner, (1906-7). From 1913 to 1917, the investigations were conducted at the George Crocker Cancer Research Institute, Director, Dr. Francis Carter Wood. During this period the chemical investigations were carried out in the laboratory of Columbia University, College of Physicians and Surgeons, in the Department of Physiological Chemistry, Director, Dr. Wm. J. Gies, and in the Pathological Laboratory of St. Luke's Hospital, Director, Dr. Francis Carter Wood, New York. The work was resumed in 1922 in the Research Laboratory of the National Jewish Sanatorium for Consumptives, Denver, Colorado, Director, Dr. H. J. Corper, and carried on there until the year 1925. Since then the work has been continued in the Laboratories of Mt. Sinai Hospital, New York, Director, Dr. L. Gross. The chemical phases since 1923 have been pursued by Prof. R. G. Gustavson, Biochemical Department, Denver University, Denver, Colorado. Some of the work has also been done in the

Medical School of the University of Colorado, Denver, Colorado, in the Department of Physiology and Embryology (1925), and one phase of the work has been made possible by the collaboration of Dr. Lemuel C. McGee, Department of Physiological Chemistry and Pharmacology, University of Chicago, Chicago, Illinois.

From the foregoing it will become evident that this investigation has required the collaboration of a large group of workers in many fields of research, and it is my desire to thank all of my co-workers and helpers to whose loyalty and effort whatever knowledge we have gained is largely due.

In the early part of this research (1915) part of the work was carried out under a grant from the Crocker Special Research Fund.

The chemical investigation has been greatly aided in the last three years by grants to Dr. R. G. Gustavson from the Committee on Sex Research of the National Research Council.

Dr. A. W. Lescohier of Parke Davis & Co., Detroit, has aided us by supplying us with both crude and refined extracts of placenta, as well as purer preparations of female sex hormone.

To Dr. C. V. Noback, Veterinary Surgeon of the Bronx Zoological Garden, we are indebted for placing rare forms of placentae at our disposal.

Many of the microphotographs were made by Dr. Sadao Otani, Assistant Pathologist, Mt. Sinai Hospital, to whom thanks are due.

At the beginning of these investigations the problem did not present itself as clearly as it does today, and much time and effort were wasted in groping in the wrong direction and pursuing fruitless leads into blind alleys. Moreover, methods of recognizing the active principle involved in the production of "feminineness" were more difficult, time-consuming and expensive than they are today since the Allen and Doisy reaction permits at least an easy qualitative recognition of the female sex hormone without sacrificing the test animal. Before this method was introduced, this field of investigation was but sparsely peopled. Since 1923 the subject has attracted innumerable workers who are elbowing and jostling each other and jockeying for position in the neck and neck race to isolate and synthesize the much desired and long sought for hormone, which is bound to relieve many of the ills from which women now suffer.

New York, May 1929

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INTRODUCTION

THE study of the female sex hormone, which is a growth substance, as I shall try to show, that has gradually become more and more specialized, until it has developed into the main factor causing "feminineness" in all dimorphous species, has now reached the critical stage in which we may hope at any time to obtain a pure substance. Such an achievement will not only be another triumph for the physiologist, endocrinologist, and chemist, from the theoretical standpoint, but will carry with it many invaluable, practical results in the treatment of diseases of women.

In this monograph there is no thesis to defend. The sole object actuating me is to present our present state of knowledge as far as is warranted by actual, proved facts. Unquestionably, as has happened in the past, modifications in hypothesis as well as in theory will be required. Unfortunately, as in every other endocrine field, much fallacy has crept into the literature and with a strange persistence, crops up again and again, no matter how often disproved and discredited. I have repeatedly pointed out that the very first attempt at organotherapy, which aroused so much widespread interest, and has had such far reaching consequences, was entirely wrong. I refer to the experiments of the then aging and senile Brown-Séquard with testicular juices, to which he ascribed a strong rejuvenating effect.

At present, a large number of investigators believe that there is only one female sex hormone to which all of the phenomena exemplifying sex may be directly ascribed. These effects embrace the estrual cycle in the lower animals and the menstrual cycle in the anthropoid group. Other workers, among whom are included Leo Loeb, Papanicolaou, Hisaw, Parkes, and Corner, have collected evidence tending to show that in addition to the ovario-placental, anabolic hormone, there is a second inhibitory substance derived from the corpus luteum at the same time that the yellow body still secretes female sex hormone. This special corpus luteum hormone regulates the rhythm of the cycle and sensitizes the uterine mucous membrane. These researches show that the corpus luteum, like the anterior lobe pituitary, secretes two different hormones simultaneously. Biedl defines a hormone as a specific substance elaborated by and in a specific organ. Minute quantities of the substance must produce definite effects similar to normal phenomena and by means of humoral (not nerve) paths.

Quite recently a most interesting and important interrelationship between the anterior lobe of the pituitary and the ovary, long suspected, has been definitely demonstrated. By means of anterior pituitary implantations Philip Smith, and by extracts, Zondek, have been able to produce activation of the infantile ovary with resultant premature puberty. By the same means they have also been able to exaggerate the lutein formation of atretic follicles, thus reproducing the ovarian condition noticed in hydatid mole and chorion-epithelioma. Steinach and Kun have succeeded in rejuvenating senile animals by means of pituitary stimulation exerting its effect on the ovary. This newly discovered interrelationship shows that the puberty stimulus originates in the pituitary and not in the ovaries. It does not explain, however, what factor stimulates the pituitary at this time. Parkes and Bellerby believe that after complete destruction of the follicular apparatus, the estrual cycle may continue. Thus another element of uncertainty has been raised.

Together with numerous collaborators, I have been engaged in these investigations for so many years and am in consequence so familiar with the situation that it may be of service to others to present a survey of our present knowledge, pointing out as well as I am able, sources of misinformation which have proved a stumbling block to many, trying to show the road along which our advances have been developed, attempting as impartially as is possible, to apportion credit where credit belongs, sifting out the wheat from chaff, thereby assembling into a comprehensible whole the many fragmentary yet interdigitating reports which have accumulated.

Quite automatically the work falls into at least two main divisions, the first one dealing with the history of the subject, the chronology of achievement, and the course of its development. It must be recognized that the preliminary phases of investigation were largely morphological. Succeeding this, physiologists took a leading hand, doing their own chemistry, with a consequent period of rather crude chemical research. In later years a fortunate combination of biological investigation, in collaboration with trained chemists, has become more general.

The second part of this monograph owes its inception to the clinician who, through his dire need, has really obliged the laboratory worker to take up and persist in this difficult and often disheartening investigation. No phase of clinical endocrinology has been more fruitful than the investigation of the female sex organs. The years have witnessed an increasing clarification of disease complexes with a consequent division into *functional* and *organic* lesions. As our knowledge increased, it has become more and more apparent how dependent the ovarian function is upon the rest of the body, and how derangement of other endocrine glands may react upon the ovary. The response of the ovary in the last analysis, however, will always be found to be of one of three types of reaction—cessation of function (in this group we may include likewise those cases in which function never was established), underfunction, and overfunction. In this branch of endocrinology, even more so than in others, empiric medication has proved not only disappointing but misleading. I am sorry to have to include in this indictment even the latest biological products which contain small and often minimal quantities of active substance.

The literature dealing with the *female sex hormone*, a name which I prefer, as all inclusive and because it does not prejudice as to the source and derivation of the active substance, has increased by such leaps and bounds that any attempt to offer a full bibliography would entail a disproportionate amount of labor as well as add unduly to the bulk of this monograph. Abel in 1926 found that for that year over 3000 titles were classified under endocrinology. This flood is increasing constantly. I have therefore confined my bibliographic references as well as my discussion, to such reports as are germane to the subject.

Among the foundation stones of this special phase of endocrinology, dealing with the female sex organs, I place such articles as that of Beard, (Born-), Fraenkel, who called attention to the corpus luteum as a gland of internal secretion; next to Halban, who in 1905, basing his deduction on purely clinical phenomena, analyzed the effect of the hormone elaborated by the placenta at least fifteen years before full experimental proof could be adduced. Leo Loeb's analyses of the sensitizing and probably inhibiting action of the corpus luteum (1905-1915) are of tremendous importance. Iscovesco (1912-1915) laid the foundation for purification methods of the active substance. In 1922 I pointed out that the follicle fluid contained the active substance. Stockard and Papanicolaou (1917-1918) rediscovered the cyclical changes in the rodent vagina, first observed by Morau and Lataste, and thus paved the way for Allen and Doisy's (1923) elaboration of a simple and easy qualitative test for the recognition of the active substance. Since then dates the speeding up of the work along these lines. The simultaneous demonstration by Loewe of Dorpat and our group, of the active substance circulating in the blood in 1925, has given additional confirmation of humoral influences in the body. This lead us to elaborate a clinical test to estimate the ovarian activity in the living human female (Frank and Goldberger). And finally, the demonstration by Philip Smith and by Zondek and Aschheim of the dominant influence of the anterior pituitary lobe upon the genital system, through its influence on the ovaries, has actually afforded proof of the long suspected and constantly referred to interaction upon each other of two glands of internal secretion.

PART I

BIOLOGY, PHARMACOLOGY AND CHEMISTRY

CHAPTER I

THE PHASES OF LIFE

DETERMINATION OF SEX

AMONG animal organisms some form of sexual reproduction is almost universal. In the fewest types parthogenetic fertilization suffices; in the vast majority of forms conjunction of a female and male element is necessary for rejuvenescence and the perpetuation of the species.

Special cells which carry on the process of reproduction are called sex or germ cells. The remaining cells of the body, eventually resulting from the multiplication of the germ cells, are called somatic cells. The question of how the sex of a new individual is determined is still unsettled.

Chromosomal Theory

The majority of biologists agree that sex is determined at the time of the fusion of the male and the female pronucleus. The chromosomes transmit this factor, the male sex containing an odd number and one less than the female. See Goldschmidt (187).

Metabolism Theory

Doncaster (100), Riddle (390), Banta (34) predicate a sexually bipotential anlage which develops either in a male or a female direction, depending on the rate of metabolism or other extraneous factors existing during the early formative stages.

Hormonal Theory

Some investigators concentrate their attention upon the nature of the sex gland which, acting upon a neutral soma (for literature see Lipschütz, 290) through the hormone it elaborates, can produce either sex, or also intermediate intersexual forms. Their attention is focused upon a less fundamental problem, namely on the changes of the secondary sex characters, either during their developmental period or reversion later on, after their full formation has taken place. I shall return to this question repeatedly.

Hermaphroditism

Certain organisms contain within themselves the essential characters of both sexes. Among the worms a condition of physiological hermaphroditism may exist, with both sex glands in an active state, coincidentally or alternately (cyclically).

A latent state of hermaphroditism may be activated under special conditions as in the fowl. Removal of the left, functional ovary and injury of the right, vestigial ovary, may be followed by development of the right gonad into testicular tissue (Domm, 98, 99; Lillie, 285).

A quantitative conception of sex is justified by the graded transitions from a virile to effeminate male; the intersex; a masculine female with all grades leading to the most feminine female. Striking instances of sex reversal resulting from disease are recorded in the literature. Moreover, experimental sex reversal has been reported (Steinach, 429, Lipschütz, 288, 291).

Very rarely true hermaphroditism has been observed in human beings (Lacassagne, 260).

It is impossible in the present exposition to more than most briefly refer to this fascinating phase of biology. Those interested in pursuing the subject may turn to monographs such as Morgan, "Heredity and Sex" (New York, 1913); Doncaster, "The Determination of Sex," (Cambridge 1914); Wilson, "The Cell"; Goldschmidt, "The Mechanism and Physiology of Sex Determination," Marshall, "The Physiology of Reproduction," (London, 1922); or to the monographic literature, of which I can refer to but a few authors such as Lillie, Moore, Domm, Ostlander in Chicago; Morgan, Wilson and their co-workers in New York; Riddle and Banta at Cold Spring Harbor; Steinach, Sand, Lipschütz abroad.

Embryonal Conditions

The Gonads

Our attention in this instance must be focused on less fundamental questions than the *determination* of sex. Whatever the primary cause may

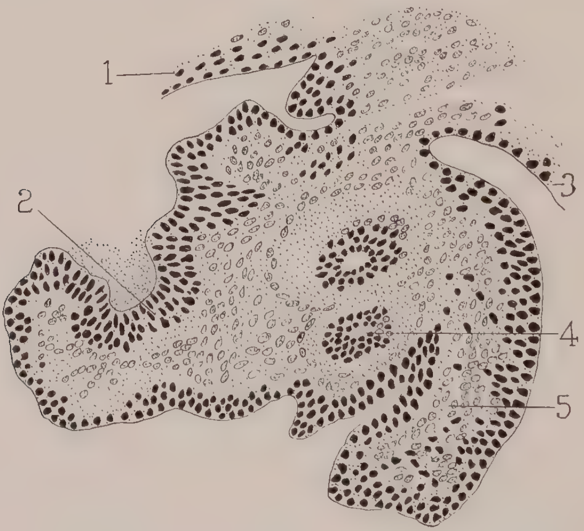

Fig. 1. Transverse Section Through Anlage of Right Müllerian Duct, 10 mm. Human Embryo.

1. Lateral body wall. 2. Müllerian groove. 3. Mesentery. 4. Mesonephric tubule. 5. Genital gland. Redrawn from Prentiss and Arey. (From Frank: Gynecological and Obstetrical Pathology, Appleton & Co., 1922).

be, a histologically neuter, early phase, during which the anlage of the gonads is laid down mesial to the mesonephric portion of the urogenital fold is noted. At this stage no recognizable characteristics of either sex are distinguishable by our present criteria. In the human embryo, at the third week of intrauterine life, sex is first distinguishable by the appearance of the *testis cords* in the male (embryo of 13 mm.). In the female embryo a characteristic *inner epithelial mass* is not recognizable until the fifth week (embryo of 50 mm.).

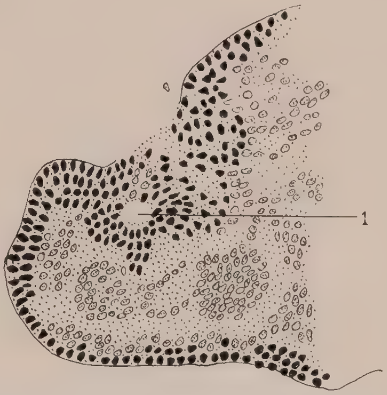

Fig. 2. Same as Figure 1 but Three Sections Caudad Showing Tubular Anlage of Duct.

1. Müller's duct. (From same sources.)

The Tubular System

The anlage of the tubular system in the human embryo is always bisexual. Both the male, Wolffian, and the female, Müllerian ducts are laid down equally, and up to a certain stage are present and develop equally. (Fig. 3) Later in embryonic life a predominance of the existing sex gland makes itself manifest by causing a predominant development of one of the tubular systems, either male or female. A coincident lagging behind of the other opposite system is physiological, but even in the normal individual vestigial remains of the tubular system of the opposite sex persist throughout life. Thus in the normal female the Müllerian system develops to the adult stage. The male, Wolffian system, is however, recognizable in well marked rests which occur in the form of separate segments throughout the length of the tubular tract, as the epoöphoron near the ovaries, as the paroöphoron in the infundibulo-pelvic ligament, as Gaertner's duct in the base of the broad ligament, cervix, and vagina (Fig. 3).

Do The Fetal Gonads Secrete A Hormone?

The question as to whether the sex glands of the fetus produce a hormone during intrauterine life has often been raised and has never as yet been clearly answered by means of experiment. We (Frank, Goldberger, and Felshin) are at present engaged in attempting to answer this question. Carlson (68-9) has demonstrated that the pancreas of fetuses function and sustain the life of the pregnant mother. The depancreatized bitch showed greater impairment of sugar metabolism after partus. Bell (38) found that the thyroid of fetuses contains hormone. Werelius (467) on the other hand, determined that the fetal parathyroids appear inactive. The scant newer literature will be found in Thomas, "Innere Sekretion in der Ersten Lebenszeit" (440). As I shall later show, a large amount of female sex hormone circulates in the maternal blood and can likewise be recovered from the cord blood of the newborn fetus (Loewe, 317). It therefore follows that the fetuses of both sexes are subjected to this female sex hormone influence throughout intrauterine life unless some special protective mechanism intervenes. It would seem that the male fetus is not harmfully influenced by this continued exposure to the female hormone though many ascribe an anti-masculine effect to the female sex hormone (see page 110). As will be shown immediately, the uterus of the female fetus does undergo stimu-

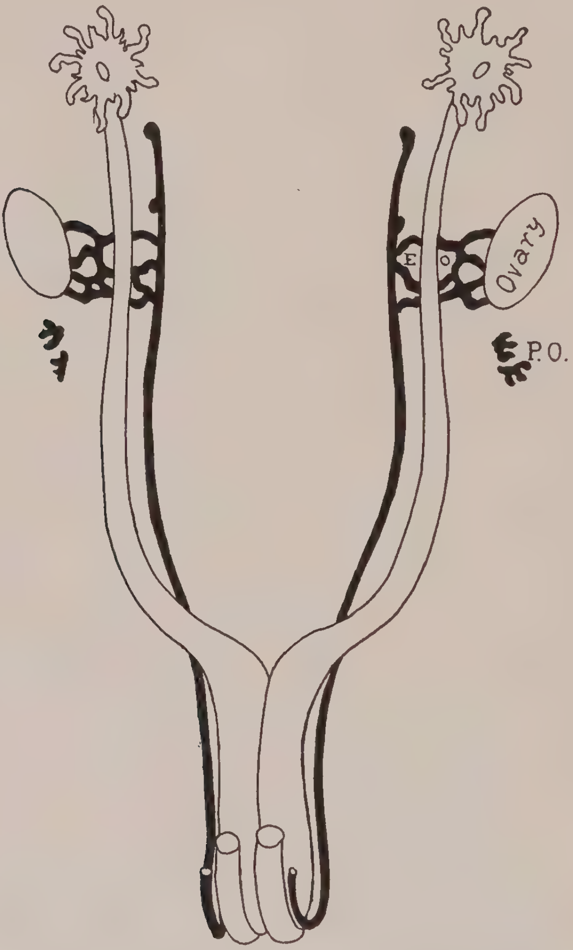

Fig. 3. Sex Organs of Embryo of Female Type.

Müller's ducts are shown in outline from fallopian tube to vulva. The male, Wolffian ducts, are drawn in solid black. E.O.—Epoöphoron. P.O.—Paroöphoron. These are remains of the Wolffian system found in the adult female. The lower portion of the Wolffian duct where it crosses behind Müller's duct may be found in the adult female in the form of Gaertner's cysts.

lation from the maternal hormone. The breasts of both male and female fetuses respond likewise to this hormonal stimulus.

As Lillie (284) has shown in cattle, monovular twins of different sexes in whom an anastamosis through the placental circulation obtains, evince malformation of the female twin, the resultant malformed fetus being known as a Free-Martin. Lillie explains the malformation on the hypothesis that the male sex hormone dominates over the female sex hormone and thus produces a typical development of the female sexual system, an anti-feminine action.

Courrier (78) has reported that injection of female sex hormone during the later stages of pregnancy into carrying mice results in the birth of female young whose sex system shows marked hyperplasia and activity in comparison with normal young. This claim has been contraverted by Parkes and Bellerby (365-6). It appears to me that Courrier's results must be based on some misconception, because when we consider the high concentration of female sex hormone circulating in the blood of, for example, the human female throughout pregnancy, it seems almost impossible to conceive of raising this level by any now known experimental means. The only possible explanation would be that in the mouse no such high hormonic blood level obtains throughout pregnancy. With the present method of testing for the hormone, it is impossible to solve this question by experiment because no sufficient amount of blood can be collected from the pregnant mouse.

Hartmann (210) reports that follicles develop and are found in the ovary before birth, reaching the stage of early Graafian follicles, and that small corpora albicantia occur in the fetal ovary. This bespeaks at least a certain grade of development of follicles with subsequent atresia. It seems quite possible to solve the question whether the fetal ovary secretes hormone experimentally, by collecting the ovaries of a large number of fetuses, extracting them and testing for female sex hormone as has been done with glands of internal secretion of fetuses. (See Frank, R. T., "Symposium on the Relation of the Glands of Internal Secretion to Gynecology and Obstetrics" (140)). We are at present engaged in an attempt to settle this query. Preliminary experiments show no greater amount of hormone in fetal ovaries than in other fetal tissues.

Comparatively large quantities of the ovaries of fetal pigs, when extracted, gave no reaction, although the livers of these same animals contained an appreciable amount of female sex hormone. Our examination extended over three different batches of fetal organs of the pig, the fetuses being graded as small, medium, and large in size. It would thus appear that the fetal ovaries do not secrete female sex hormone.

Early Post Partum Conditions

Neonatus

As previously mentioned, the uterus and breasts of the newborn female child may show activation and are then larger and better developed than those of a child of one year of age. The breasts of the newborn male (Bayer, H., 36) as well as those of the female, may show this marked hyperplasia, producing a button-like projection upon which is seated the minute nipple. From the breasts of the neonatus of either sex, colostrum may be expressed in a fair percentage of cases (Hexenmilch of the Germans, witchs' milk of folklore). Halban (200) as early as 1905 correctly interpreted this interesting condition, ascribing the stimulus to substances secreted by the placenta. During intrauterine life, the female sex hormone, as we now call the substance secreted and circulating in the blood, is absorbed by fetuses of both sexes. In the female, both the breasts and the uterus are activated. In the male the breast and sometimes the prostate (Schlachta, J., 402) respond. After expulsion of the fetus and the consequent withdrawal of the stimulus (just as in the adult mother), the breasts secrete (Frank R. T., 136). Since Halban's theoretical explanation, numerous experimental proof has been adduced to substantiate his contention (Basch, 35; Frank and Unger, 166; Frank and Goldberger, 157).

The excitation exerted upon the uterus of the new born by the maternal female sex hormone regularly produces hyperplasia of muscle and endometrial lining. In certain instances the stimulus may reach such a degree that after separation from the mother, an abrupt retrograde catabolic involution occurs, which is strictly comparable to the menstruation of the adult (Zappert, 478). Hemorrhages from the uterus occur in 2.5 per cent of female children, appearing in the vagina and at the vulva, lasting for several days (Zacharias, 476). This condition which, under the microscope, is recognizable by specific changes in the uterine mucosa, must not be confounded with genital hemorrhages occurring coincidentally with hemorrhages elsewhere in the body of the newborn as the result of sepsis or a general hemorrhagic condition.

PREPUBERTAL STATE

From the early weeks of life onward until the approach of puberty, although the female child in many ways gives the picture of an asexual or neuter state, evidence is at hand to show that a continuous though not large secretion of hormonal substance from the gonad occurs (Fehling, 112, pelvic contour). Anatomical evidence is furnished by the slowly increasing number of growing primordial follicles, which reach early Graafian size and development but then undergo atresia (Hartmann, 210). Considerable evidence obtains that atretic follicles, by virtue of their proliferating granulosa layer, are likewise able to secrete small amounts of hormone sufficient to control trophic growth. Quantitatively follicle ripening is still insufficient to initiate puberty (Frank, 143).

PUBERTY

In the human female the approach of puberty manifests itself by well marked somatic and psychic changes. In some of the lower forms special changes may occur characteristic of a given species. In the rat, for example, and this will be referred to later, prepuberty shows itself by the opening of the vagina.* The first estrus may but need not follow shortly thereupon, ordinarily in three to five days. In this preliminary discussion, however, I shall focus my attention mainly upon the human subject.

Secondary Sex Characters

As was emphasized in discussing the determination of sex, every individual appears capable of developing from an intersexual model toward maleness or femaleness, depending upon the character of the hormone secreted by the gonad. Those characters typical of the human female are known as the human secondary sex characters. At the period at which the adolescent verges toward puberty it is well recognized that certain changes in the voice, consequent to changes in the framework, cartilage, and musculature of the larynx occur. At this time likewise accumulation of fat in the region of the mons, the nates and the mammary glands take place, which are characteristic of the female. In both sexes axillary and pubic hair appears at this time of life. In the female the pubic hair terminates in a concavity turned upward, while in the male the hair line is triangular with its apex approaching the umbilicus. The female breasts develop with cup-shaped configuration,increase of nipples and accentuation of the areola. Even before the onset of puberty the pelvis shows a broadening with consequent broader intertrochanteric measurements and resultant changes of the pelvic bones, which eventually produce the roomy female pelvic basin with obtuse subpubic angle, widely separate ischial spines and tubers. These various phenomena, together with psychical heterosexual inclinations changes in demeanor (such as modesty, shyness) are classified under the general term of secondary sexual characters. As will be shown hereafter in certain individuals of a neuter type, these characteristics never develop. In rare instances, in female adults, due to disease, a change from the normal feminine secondary sex characters toward a neuter type, or more rarely an actual transformation toward a masculine type, may occur. For the many interesting details the reader is referred to the monograph of Tandler and Grosz (437).

Normal Puberty

In the human female, besides the general signs of pubescence just referred to, normal puberty usually culminates in the first menstruation.

Puberty Without Menstruation

That puberty can occur without the appearance of menstruation is well illustrated by a small group of women, of whom I have observed three, who in spite of never having menstruated, have proved fertile and have born one or more children (see page 218).

In another more numerous group, to which I shall also refer again in the second portion of this volume (page 257), menstruation can never occur because of the absence of vagina and absence or hypoplasia of the uterus, thus effectually precluding any menstrual flow. These individuals, however, may show female characteristics as evidenced by their secondary sex characters, including their psyche. In addition, by our method of blood examination, we have been able to demonstrate ovarian activity by noting the repeated cyclical accumulation of female sex hormone in the blood of a number of these handicapped individuals (page 257). Under these conditions we feel justified in classifying such individuals as *females* instead of malformed males or neuters.

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* Castration of very immature rats does not prevent opening of the vagina at the usual age.

INDUCTION OF PREMATURE PUBERTY

1. *By Injection of the Female Sex Hormone*

Many investigators, including the pioneers, have shown that immature animals can be made sexually mature by injections of active hormonal extracts obtained from the ovary or placenta (Aschner, 27; Schickele, 400-1; Fellner, 114; Frank and Rosenbloom, 165; Allen and Doisy, 7). Frank, Kingery, and Gustavson (164) have also shown that after the injections of the active substance have been stopped, some of the immature rats may remain "adult" and continue ovulating cyclically.

Immature castrates likewise can be made precociously mature but, as a matter of course, relapse to a neuter condition as soon as the injections are stopped.

2. *By Means of Anterior Lobe Pituitary Implants and Extracts*

The intimate interrelationship between anterior pituitary and the ovaries is becoming more and more manifest. The anterior lobe has been called the motor of the gonads. Therefore it is necessary to describe these relationships in the present monograph with some detail. The pituitary influence manifests itself most strikingly at puberty. It is for this reason that the entire subject of anterior lobe effects is taken up here.

Quite recently Philip Smith (421) as well as Zondek and Aschheim (486-7) have demonstrated that puberty is dependent upon secretion of the anterior lobe pituitary. Implantation of fresh anterior lobe pituitary substance or injection of active extracts of the same are rapidly followed by estrous phenomena in the immature experimental animals.

In this instance the activation is indirect, as, after a short latent period, the resting infantile ovaries enlarge because numerous follicles mature and rupture.

> In 1911 (Frank and Unger, 166) I injected extracts of hypophyses intraperitoneally into immature rabbits, without noticeable results. Had rats been used, puberty signs would have been recognized. This is one of the many instances where success or failure depends on the selection of the right species of experimental animal.

According to Fellner (118) and to Brouha and Simonnet (61) puberty may likewise be induced in immature castrates by means of anterior lobe pituitary extracts. Their results remain unconfirmed, especially as we (Frank and Goldberger, unpublished) have repeatedly attempted to obtain such effects without success.

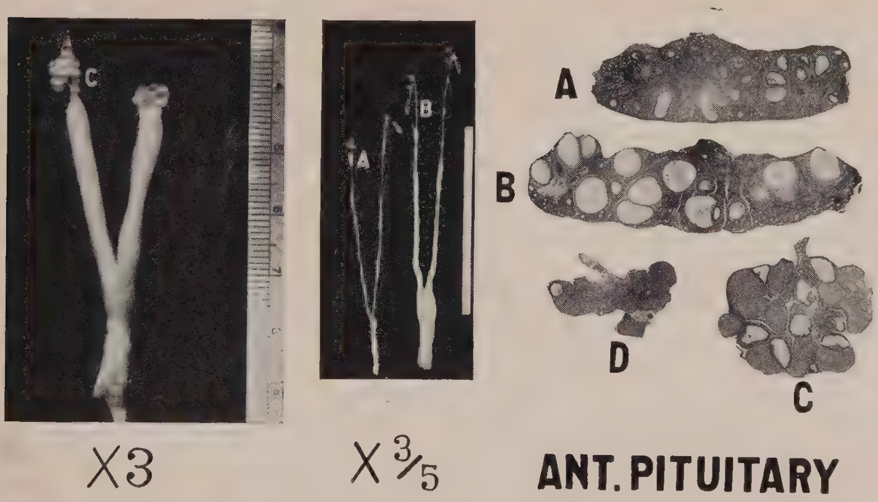


Fig. 4. Anterior Pituitary Stimulation of Ovaries and Genital Tract by Means of Implantation of Dog's Anterior Pituitary Repeated Daily for 8 Days.

A. Control rabbit.
Black A. Low power photomicrograph of ovary of control.
White A. Shows ovaries and tubular tract of control rabbit.
Black B. Shows low power of ovary of stimulated rabbit. Note the numerous cystic follicles.
White B. Great stimulation of the tubular tract of this rabbit and also macroscopic increase in size of ovaries.
Black C. Hypertrophied ovary of stimulated rat containing large number of corpora lutea. Low Power.
White C. Macroscopic appearance of stimulated ovary of rat.
Black D. Corresponding unstimulated ovary of normal rat. Low Power.

The anterior pituitary experiments performed by us after the directions of Brouha all proved negative. The following are a typical series:

Fresh cattle pituitaries; anterior lobes dissected out; ground in a mortar with sand. Three volumes acetone added. Stood for several days. Evaporated on water bath. Residue taken up in olive oil.

1 c.c. = 50 mg. anterior lobe substance.

4 immature female mice from same litter.

M. 6 gm. injected 1 c.c.; vagina opened in 6 days; spread positive 17 days later (23rd) weight 18 gm.

M. 8 gm. injected 1.5 c.c. died.

M. 6 gm. control, vagina opened 18 d., spread positive 3 d. later (21 st d.) weight 17 gm.

M. 8 gm. control, vagina opened 19th d., spread 0 at 16 gm.

Castrate mature M. 3 c.c., smear = 0

Castrate mature M. 2 c.c., smear = 0

A second batch 49 mg. = 1 c.c.

Immature M. 0.5 c.c., vagina opened 16 d. 9.5 gm., smear negative for 33 days.

Immature M. 1 c.c., vagina opened 18 d. 9.5 gm., smear 0

Castrate adult M. 0.1 c.c. = 0

Castrate adult rat 1. c.c. = 0

Castrate adult rat 2.5 c.c. = 0

We are therefore in no way able to confirm their findings although their course of experimentation was accurately followed.

Putnam (380a) agrees with me in that he was unable to repeat Brouha's or Fellner's positive results with anterior pituitary extracts in castrate rats.

In preparing maturity inducing anterior lobe extract he found that Spaul's (427a) directions for making an acid aqueous extract were good.

Putnam (380b) by daily injections of special anterior lobe extract was able to produce overgrowth, splanchnomegaly, enlarged acra and polyphagia in a bulldog. This closely approaches an induced acromegaly.

The most recent work has shown that anterior lobe extract produces an increase of the blood cholesterol, but only indirectly through the ovary, as the effect cannot be obtained in ovariectomized animals (Reiss and Langendorf, 385a).

Using the Aschheim-Zondek technic (see page 19) Squier and Wertheimer (427b) found that no evidence of the presence of anterior lobe pituitary secretion could be demonstrated in the cerebrospinal fluid of female dogs.

Lehmann (278) 1927, reports his ability to cause the pituitary of castrated rats to return to normal by means of injections of female and male sex hormone, each acting only sex specifically.

Baniecki (33) 1928, reports having succeeded in producing the anterior lobe pituitary changes characteristic of pregnancy in female guinea pigs by means of injecting "ovarian" hormone. Male pigs proved refractory

On the other hand, Berblinger (40) 1921, reported "pregnancy" hypophysis as the result of injecting placental extracts into male rabbits.

Engle (104a) by daily transplants of anterior pituitary into mice and rats, was able to induce superovulation with resultant 20–28 ova into each uterine tube. In the mouse pregnancy then resulted in the formation of 19–29 fetuses.

Rumph and Smith (397a) found that anterior pituitary secretion could be demonstrated in pig's fetuses of 28 cm. or larger.

3. *By Means of the Maturity Inducing Factor of the Anterior Pituitary*

Still more recently Evans and Simpson (110) have fractioned anterior pituitary extracts into two components. The one they claim is growth producing, the other a maturity inducing factor. The growth producing substance is said to be used up until the soma no longer requires it, whereupon the maturity inducing substance gains the upper hand and is able to initiate ovarian activity. These investigators claim that the one substance is derived from the acidophil (eosinophil), the other from the basophil cells. If this almost too beautiful and exact interdigitation and neutralization of synergistic substances accounts for the phenomena as described, it will prove to be a most wonderful mechanism.

> The influence of the anterior lobe pituitary secretion is not limited to the time of puberty. During pregnancy a great increase of pituitary secretion occurs in the blood and urine. This fact has been utilized by Aschheim and Zondek (24) for a biological test of pregnancy (p. 19).
>
> As the indirect influence of the pituitary on the ovaries is so manifest, the possibility of constant pituitary control, including the production of the periodicity of the sexual cycle, cannot be disregarded entirely. This, particularly since Parkes (361–2) insists upon the continuation of periodicity after all ovogenic tissue (follicle, corpus luteum) has been destroyed by the X-ray, (p. 124).
>
> It is not known whether at the time of senescence the pituitary secretion diminishes. This may be so, for senile ovaries can be reactivated by pituitary injections or implants. (432).

The absurd assertion that I considered the anterior lobe pituitary influence "*supposititious*" was first made in a widely distributed pamphlet of a pharmaceutical house.

> It was due to the deliberate, or accidental selection of a single phrase, which in no way applied to the *influence of the pituitary gland*, but to inert *desiccated anterior lobe pituitary extracts.* (Frank, 143). My previous work, as well as my remarks, dealt solely with the then obtainable market preparations. In this connection it is worth recalling that Cushing (87), who had originally utilized pituitary extracts in his clinical work, later receded from this position and realized the impotence of such preparations.
>
> Yet to my surprise, Novak (349) has read this same meaning of "supposititious" into my statement. Therefore when he writes that "*the assertion of Frank that the pituitary has nothing to do with the causation of uterine hypoplasia*" and later "*it would be absurd to argue that the existence of such a relation is made doubtful by the mere fact that pituitary feeding does not give results in case of underdevelopment of the generative organs*," his deductions are erroneous, for he puts words and opinions in my mouth which I never entertained. My remarks applied solely to Goetsch's experiments which have been disproved not only by me but later also, by Sisson and Broyles (416) and by Kross (257).

4. *By Means of Pathological Growths*

As is well known, premature puberty is observed in the human female as a result of various pathological factors such as pineal tumors, adrenal growths, as well as ovarian neoplasms. In a certain number of instances

(J. Lenz, 279) (Reuben and Manning, 387) no demonstrable gross lesion could be found. Binz (46), as well as Frank and Gustavson (unpublished case of Eastlake and Ingraham) have demonstrated the presence of an active substance in the ovarian tumors removed from children with premature sexual ripeness (see page 237). A full account of the adrenal effect will be found in Jaffé and Tannenberg (245). If the cause is ovarian, menstruation appears with the other signs of puberty. In cases of adrenal tumors, the secondary sex characters develop (often virilistic in type) but menstruation does not occur.

Effect of Castration

Puberty does not take place if castration is practiced upon an immature animal. The resultant type is a neuter (eunuchoid) form. (Consult Tandler and Grosz, 437). If the gonadectomy is performed after the secondary sex characters have developed, the permanent changes such as bone growth remain, but other less stable characters, such as hair development, fat distribution, psyche, may regress to the neuter state. I know of no early castrations performed on children who survived sufficiently long to show these striking effects in the human.

Maturity

In the human female the period of sexual maturity begins at the 9th to the 16th year, depending upon race and habitat (105), enduring from then on until the onset of the menopause, which occurs on an average from the 42nd to the 52nd year (105) in temperate climates. In adult women a cycle occurs at intervals of from 21 to 35 days, with an average of from 26 to 30 days. The most striking and readily recognizable symptom is the monthly bleeding from the vagina—menstruation.

Menstruation appears to be limited to the primates. It is found with regularity in certain monkeys (Heape, 213–4; Halban, 199; Corner, 74; Hartman, 208; Allen, 4). The bleeding seen during the estrous cycle in lower species, namely the bitch, sow, cow and mare, is quite different from menstruation, for it is noted at the height of estrus, during the anabolic phase of the cycle. Menstruation, on the contrary, signifies the onset of the katabolic phase and is often associated with destruction of the superficial layers of the endometrium.

As our detailed knowledge of the minute changes which take place during the estrous cycle, has been obtained from animals, it will be necessary to discuss the question from the standpoint of comparative anatomy and physiology in considerable detail, but this will be done somewhat later, after having laid down in general outline the phenomena observed in human beings.

In the average human female, some 360 menstrual cycles should occur during the 30 years of sex life, unless the rhythm is interrupted by pregnancy. This may signify at the most 360 full maturations of Graafian follicles with resultant ovulation and corpus luteum formation. In the average fecund female, under present conditions of civilization and economic stress, at most 3 to 10 pregnancies may be expected during the entire period of sexual maturity, leaving 350 cycles incompleted or abortive.

The subdivision of the cyclical variations into an infertile or abortive cycle and into a fertile cycle, as done by Frank and Gustavson (162) (1925) appears warranted. To what extent the direct comparison of menstruation to abortion of an unfertilized ovum is justified will be left open for the moment.

If an ovum is normally fertilized in the tube, then descends into the uterus and is imbedded, pregnancy occurs (fertile cycle). In the human female, gestation lasts approximately 278 days and ends in labor and birth.

Post partum there is an involutionary (puerperal) period during which the enlarged Müllerian structures (vagina, uterus, and tubes) regress. The puerperium merges imperceptibly into the lactation period. Lactation may put a stop to ovulation or ovulation may be resumed during this time (see later). After lactation is terminated, the cyclical changes reappear.

MENOPAUSE

In all species at a certain age, the cyclical phenomena gradually become less marked and eventually cease. Whether this cessation is due to exhaustion of the follicles or whether another mechanism comes into play, has not been determined. The fact that Steinach and Kun (432) have been able to reactivate senile females by implantation of anterior lobe pituitary fragments speaks for a more complicated mechanism than mere cessation of function due to exhaustion of follicles.

With the menopause, involution of the Müllerian system takes place. The vulva and breasts are affected in a similar manner, and some of the less constant secondary sex characters, especially the hair, as well as the voice and psyche may undergo demonstrable changes verging toward a neuter type.

The Aschheim-Zondek Pregnancy Test for Anterior Pituitary Hormone (*24*). This test is so often referred to in the text that a brief outline is here given.

Five immature female mice, weighing between 6 to 8 g. (3 to 4 weeks old) are injected with the slightly acidified morning urine of the patient, in quantities of 0.2–0.25–0.3–0.3-0.4 respectively, six times (the first day 11 A.M. and 5 P.M.; second day 10 A.M., 2 and 5 P.M.; third day 10 A.M.). The mice are killed on the morning of the fifth day, counting from the day of the first injection (as Monday to Friday). At autopsy the presence of *blood* spots in the enlarged ovaries signifies a positive reaction-pregnancy. The opening of the vagina and positive vaginal smears are not diagnostic.

CHAPTER II

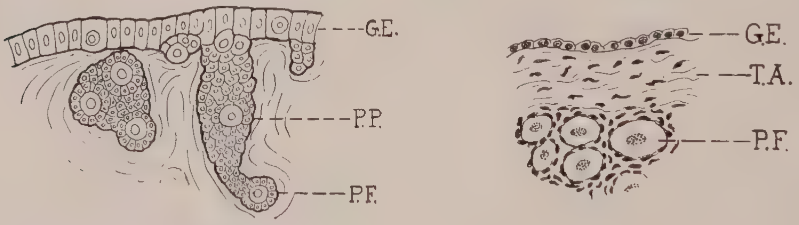
ANATOMY OF THE GENITAL TRACT

THE OVARY

THE ovaries are glands of both external and internal secretion. The ova represent an external secretory product. The internal secretory portion of the gonad is elaborated by the follicle epithelium. According to some (Aschheim, 20) the theca interna of the follicles, according to others, the interstitial gland (Wallart, 465; Seitz, 413) likewise produce a hormone. An excellent monograph containing the normal histology of the genital system is that of Moraller and Hoehl (341). It is almost impossible to separate description of the anatomy from that of the physiology of the genital organs, because of the constant changes which take place during maturity.

Genesis

The ovary is developed from the coelomic epithelium in the region where a ridge mesial to the mesonephron appears. The site of the origin of the primary germ cells has never been fully settled. According to Fuss (172) these primary germ cells wander in through the mesentery to reach the anlage of the gonad. According to Swift (435), in the chick, the primary germ cells can be traced from an extra-embryonal origin, that is from outside the actual periphery of the embryo down to their final site. This theoretical question, may have a very practical bearing on the origin of complicated dermoids within the chest and along the base of the mesentery. It is likely that early blastomeric cells of full potentiality to develop into all tissues may be arrested in their course to the future ovary or testis and form dermoids in these localities.



5A 5B

Fig. 5A. Schematic Drawing of Fetal Ovary Showing Origin of Primordial Follicles.
G. E.—Germinal epithelium. P. P.—Pflüger's Plug growing downward into stroma of ovary. P. F.—Primordial follicle still attached to plug.

Fig. 5B. Section of Ovary of Newborn.
G. E.—Germinal epithelium. T. A.—Tunica albuginea. P. F.—Primordial follicle.

The most widely accepted explanation of the origin of the primordial ova is that of Pflüger (375) who has traced the down growth of the germinal epithelium covering the anlage of the gonad into the deeper layers. These so-called Pflüger's plugs are then said to break up and form both the ovum proper as well as the single layer of follicle epithelium which surrounds the primordial ovum. If this is correct the ova are formed by the lining layer of the cœlomic cavity.

Gross Anatomy

There are two ovaries in mammals, in the embryo situated immediately below the kidneys, whereas in the adult they have descended into the pelvis. In the human, the ovary projects with nine-tenths of its area free into the peritoneal cavity, covered merely by the single layer of germinal epithelium, the peritoneal coat stopping abruptly at the hilum in the form of a clearly marked white line.

The ovary is divided into a superficial or cortical layer and a deep or medullary layer.

Cortex

The cortex is further subdivided. Its most superficial layer is the single, low cylindrical *germinal epithelium*. Next comes an ill-defined matted layer consisting of connective tissue with occasionally unstriped muscle fibers, known as the *tunica albuginea*. Below this is a vascular *stroma* containing a large number of follicles. The larger maturing follicles will be found toward the medulla having grown downward, until they reach some size. After their diameter has reached one-half centimeter, it is noticed that further growth and enlargement of the follicle takes a centrifugal direction and that the completely mature follicles eventually project well beyond the surface of the ovary.

In the mare, there is a special mechanism for facilitating ovulation, namely a central incisure known as the ovulation fossa.

Medulla and Hilum

The center of the ovary is occupied by many convoluted arteries and veins, as well as lymphatics and nerves, which enter from the broad ligament through the hilum. Certain nerve ganglia and a variable amount of unstriped muscle are also found. In addition a number of vestigial remains, which are of no interest in the present connection, are found in the medulla.

At Birth

At birth the ovary contains primordial follicles in huge numbers, which have been variously estimated at 3,000, 30,000, and even 100,000 in the human ovary. In addition at least a few growing Graafian follicles are usually encountered (Runge, 398; Hartmann, 210).

The Question of Postnatal Ovogenesis

The question has been frequently raised whether new formation of primordial follicles and ova ceases at birth. This question has been variously answered. Kingery, in 1917 (252) found ovogenesis continuing in the white mouse, at least to the 45th day, when puberty set in. Arai, 1920, (18) showed that, in the mouse, new formation of ova took place until the end of the first year. Recently Allen, 1923, (3) has claimed that in the mouse ovogenesis takes place cyclically with each estrual cycle. Papanicolaou (353) reports that in the guinea pig whose thyroid has been treated with radium, postnatal, new formation of ova takes place.

The possibility of such formation is of importance in connection with the claims of Parkes (362) who states that after all follicular structures have been destroyed and made to involute by means of X-raying the ovaries of adult mice, cyclical phenomena (determinable by the change of vaginal spreads, (See page 124) nevertheless continue. The same author claims (360) that mice rayed *in utero* or at birth nevertheless become cyclical about the time of puberty. This is quite contrary to what I have observed in the human female in whom X-ray castration was produced. In them the blood cycle abruptly or gradually ceases as the effect of the X ray becomes manifest (page 252).

Wester (468) quotes all the available literature on postnatal formation of ova. For the sake of completeness I append the more important of his references:

Wagener. Arch. f. Anat. and Phys. 1879. Found new ova during estrus of bitches.

Van Beneden. Arch. de Biol. 1: 1880. In adult bat.

Balbiani. Leçons sur la Génération des Vertébrès, 1880. In adult woman.

Harz. Arch. f. Mikr. Anat. 1883. In young cattle and cats. Formation of ova from germinal epithelium. Not in older animals.

Harriet E. Lothrop. Dissertation, Zürich, 1890. In scar of freshly injured ovary found proliferation of ova.

Von Ebner. Köllicker's Handbuch der Gewebelehre, 3: 1902. In adult women.

Stoeckel. Arch. f. Micr. Anat. 53: 1899. In adult women found new formed ova.

Lane-Claypon. Proc. Royal Society, 1906, vol. 27. In pregnant rabbit new formation of ova occurs from the interstitial cells, which she regards as potential ova.

Von Winiwater and Sainmount. Anat. Anz. 1908, Vol. 32; also Arch. de Biol. 1909, Vol. 24. Cats of various ages; new formation just before puberty.

THE OVARY FROM BIRTH TO PUBERTY

In addition to the gradual descent into the pelvis to which no physiological importance can be attached,* there is a very slow increase in size of the ovaries. They appear to lag behind the general growth of the body.

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* Retention of the testis within the abdomen, on the other hand, entails grave consequences as the male gonad is sensitive to the increased intra-abdominal temperature.

(See Fig. 58, page 202). Evidently some trophic influence is exerted by the infrequent and not complete maturation of follicles, for a gradual differentiation of the entire body can be noted even before puberty occurs. Prochownik (380) found a corpus luteum in a child three years of age but this must be an exceptional occurrence.

Not until the onset of puberty does regular and well-marked increase in the rapidity of ripening and in the full maturation of follicles take place. This, as has been shown, is not a primary ovarian effect, but dependent on the activity of the anterior pituitary lobe.

MATURITY

In the life span of the average healthy woman, the period of sexual maturity lasts during 30 to 35 years. Throughout this extended time, at most 360 to 420 ova mature completely and rupture. Innumerable ova, on the other hand, undergo atresia. I emphasize this fact in order to show that atresia, which signifies the maturation up to a certain, but not to a complete degree, of numerous follicles and their gradual involution, plays an important rôle in supplying large amounts of active female sex hormone to the body. The full maturation and consequent cyclical phenomena probably, however, are due to the follicle and corpus luteum.

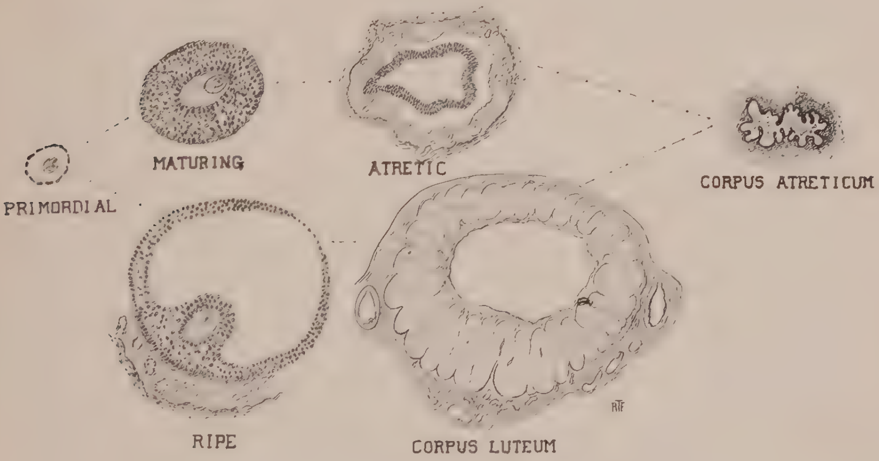

FIG. 6. LIFE HISTORY OF THE OVARIAN FOLLICLE.

All follicles start as primordial follicles. In the lower row are shown the ripening (Graafian) follicle, its successor after ovulation, the corpus luteum, and its final stage, the corpus atreticum. Above is shown the partially maturing follicle with atresia, ending likewise in the corpus atreticum. Only 1 out of every 300 follicles fully ripens. The other 299 become atretic.

The cyclical phenomena, which have been briefly referred to before, in the human as well as in other mammalian females can consist of two types of cycles. In the human the more frequent type is the infertile or abortive cycle, in which impregnation has not taken place. The fertile or pregnancy cycle, after all, occurs only from three to ten times in the average life history of a woman.

Infertile or Abortive Cycle

It is unnecessary in a monograph of this character to go into minute details of morphology. They can be found in many textbooks. I have described them repeatedly in some of my articles (Frank, 134 and 138) with full references to the voluminous literature.

At cyclical intervals a primordial follicle shows signs of growth, matures, forms a Graafian follicle with cavity, discus and ovum.* This follicle grows, projects above the surface of the ovary, and ruptures. The time of rupture is called the time of ovulation. The stigma, or point of rupture in the collapsed follicle, soon closes; the cavity of the follicle fills first with blood, which is later invaded by connective tissue. The follicle epithelium, or granulosa layer, rapidly multiplies; its cells enlarge, containing more and more lipoid, which is of yellow color. In the bitch the granulosa cells may multiply and show lutein changes even before ovulation occurs. (See Fig. 2 of the article on the "Function of the Ovary," Frank, 135). The resulting structure, which started as the simple ovular sac, is now called the corpus luteum. The outer layer of the corpus luteum is divisible into two strata: the inner, a narrow connective tissue stratum called the *theca interna*, which also shows lutein changes during the formation of the yellow body, and an outer vascular, almost cavernous layer, called the *theca externa* which merges with the surrounding ovarian stroma.

The corpus luteum then is a temporary structure formed by the ovisac and functions as a temporary gland of internal secretion. This gland is composed of large, pale, lipoid-filled lutein cells. Between these cells are thin-walled capillaries, growing in from the theca externa, which permit the internal secretion to reach the circulation. The corpus luteum of the infertile, abortive or menstrual cycle has a short life. Its vascularity is well marked only for at most two weeks after ovulation. Then involution and obliteration rapidly take place, first marked by obliteration of the capillaries, next by disintegration of the lutein cells, and finally by invasion and substitution of the lutein cell by the stroma cells. For a considerable time the involuting corpus luteum is still distinguishable as a pale yellow connective tissue scar called the *corpus albicans*.

To recapitulate, the life history of the follicle in an infertile cycle consists of the primordial stage, the stage of early maturation, the Graafian stage, rupture (ovulation), formation of corpus luteum, and corpus albicans.

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* Lack of space forbids any description of the changes in the ovum during maturation and preceding ovulation.

The Fertile or Pregnancy Cycle

The early stages of this and the preceding or infertile cycle are identical. If, however, after ovulation has taken place, the ovum is impregnated and embeds in the uterus (or even in an abnormal locality such as the tube) the resulting corpus luteum is larger and more persistent. The yellow body persists throughout pregnancy. In the human female distinct signs of a gradual loss of function appear as early as the fifth month, but full involution and obliteration do not become manifest until after birth of the child.

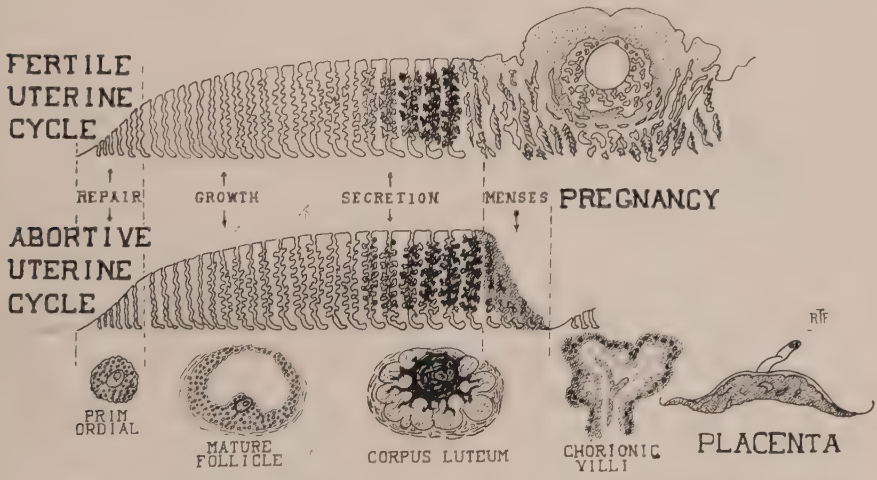


Fig. 7. Diagram of the Sex Cycle in the Uterus and Gestational Gland of the Normal Woman.

1. The fertile cycle. 2. The infertile uterine cycle produced by ovarian action. (Modified from Schröder). The various stages of the gestational gland beginning with the primordial follicle, ending with the corpus luteum in the abortive cycle and with the placenta in the fertile cycle, are shown.

As soon as the chorion ectoderm forms about the embryo, from the earliest stage of primary villi on, female sex hormone is produced. We have obtained a positive reaction from a 3–4 weeks human pregnancy, the earliest at our disposal. From then on, an increasing amount of female sex hormone can be extracted, a human placenta at term containing up to 2000 R.U. (See page 119).

As the follicle, corpus luteum, and placenta elaborate the same hormone and by their continuity of action maintain the high hormone level in the blood which appears necessary for the successful continuation and termination of pregnancy, we (Frank and Gustavson, 162) have called this triad "*The Gestational Gland*". Among others, Loewe and Voss (317), Loewe (307), and Brouha and Simonnet (60) concur in this interpretation.

The Corpus Luteum

Distribution

So much attention has been accorded to every question connected with the yellow body that it will be necessary to consider it in somewhat greater detail, in order that the reader may be in a position to appreciate the frequent references made in regard to it. This organ is the only transitory gland of internal secretion. Its existence in the infertile cycle is short, but its life is much prolonged in the fertile cycle. The corpus luteum occurs in a primitive form even in fishes and birds (Novak and Duschak, 1923, 350). It is well developed in all mammals. This includes the monotremes in which the intrauterine stage of pregnancy is very short, the stage of existence of the immature young in the abdominal pouch (marsupial sac) being more prolonged (for example, *Dasyurus*, Sandes, 399), and the opossum (Hartman, 207). Nevertheless the corpus luteum of marsupials persists throughout lactation.

Derivation

The derivation of the functional cells of the corpus luteum has given rise to innumerable controversies. Bischoff (47), as well as Sandes (399), have adduced evidence that the lutein cells are derivatives of the granulosa. Limon (286) and John G. Clark (71) derive them from the theca interna cells. Still others, I may instance Gatenby (173), derive them from both of these sources. For the literature, I refer the readers to my article (138) in which the subject is taken up in detail from every aspect.

The Development and Life History of the Corpus Luteum

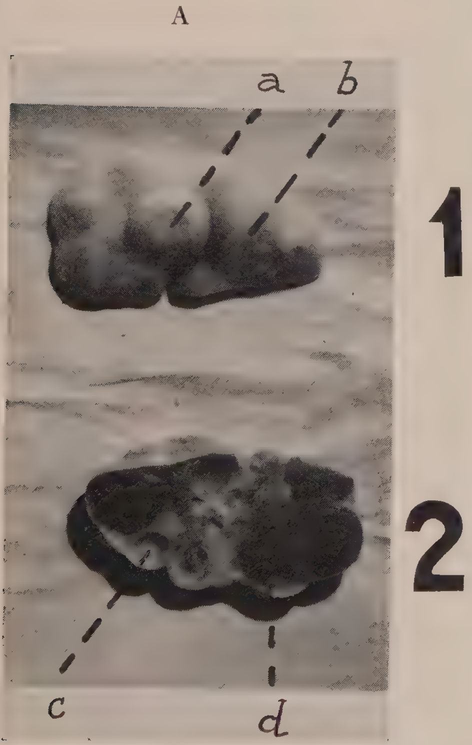
Macroscopically, the first stage is formed by the collapsed follicle. Hemorrhage or serum exudation into the follicle cavity is followed by solidification (gel stage), the red fresh corpus luteum. Not infrequently in the human being a cystic central cavity develops. The redness gradually is replaced by a distinctly yellowish tinge as the central clot is decolorized and the lutein cells acquire yellow pigment (lipochrome). As involution progresses, the corpus luteum becomes harder, less succulent, and yellowish gray in color until it gradually merges into the stroma of the ovary (corpus albicans). On cross section a characteristic yellow crenated border is readily recognizable and characteristic.

Microscopically, the process of growth and subsequent degeneration can be divided into four periods—proliferation, vascularization, ripeness, and regression (Robert Meyer, 335–6–7).

> *Proliferation.* At this early stage the theca interna cells are already separated by capillaries, and in many instances outnumber the granulosa cells in mass and prominence. The granulosa–epithelial cells, become stratified and increase in size. The follicle cavity either is empty or contains serum and blood (Fig. 9).
>
> *Vascularization.* The granulosa cells have increased to such a degree that they are the most prominent component of the follicle

sac. They are large, epithelial and cuboid in character, each containing a large spherical, faint-staining nucleus. The capillaries are beginning to enter between the close packed lutein cells. The theca interna remains unchanged and consequently by comparison has greatly receded in prominence.

Ripeness. The granulosa lutein cells have increased so much in size and number that the follicle cavity is able to contain them only by an infolding process, resulting in the production of numerous folds. This increases the surface and forms a typical crenated margin to the lutein layer, macroscopically recognizable, as previously mentioned. Between these folds, well marked septa arise through which the main blood vessels enter the new-formed gland, and which septa also harbor the theca interna cells. The theca externa forms a very vascular almost cavernous outermost layer. At this stage the struc-



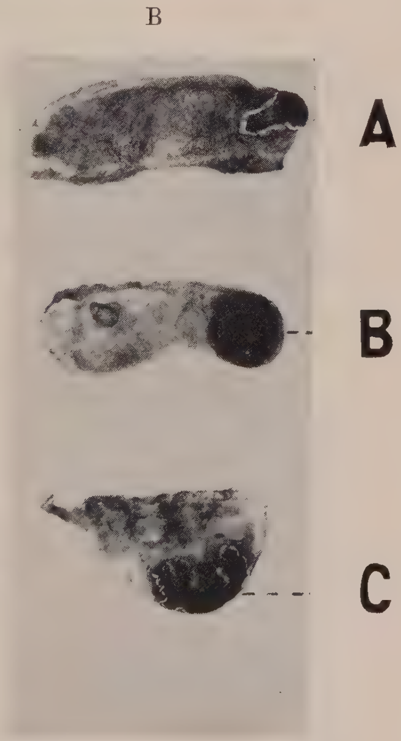


Fig. 8A. Ovary of the Hog.

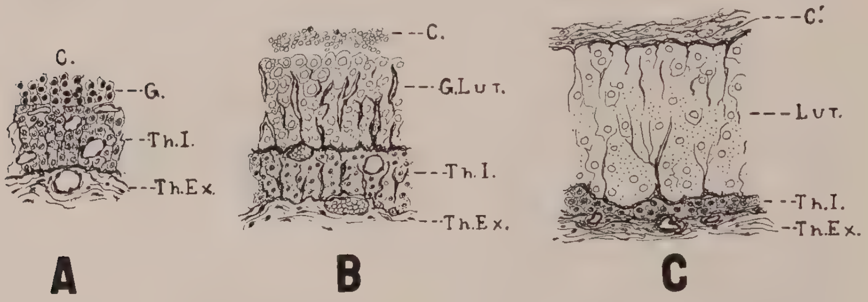
1. Outer surface: a. Follicle; b. Corpus luteum.
2. Cross section of same ovary: c. Cavity of follicle; d. Fresh corpus luteum, hemorrhagic stage.

Fig. 8B. Human Ovary.

A. Freshly ruptured follicle. B. Early corpus luteum. C. Mature corpus luteum.

ture of the corpus luteum very closely resembles that of the fascicular layer of the adrenal gland.

Regression. The color of the gland is bright yellow. The lutein cells show marked vacuolation. The capillaries between them are narrowed and in many instances obliterated. It is to this stage that I shall later refer in a different connection. At this time the lutein cells are still secreting an active substance and are storing it, temporarily, because their capillary path of excretion has ceased to function.



Figs. 9, 10, 11. Diagrammatic Outline of Growth of Corpus Luteum.

Fig. 9—Follicle immediately after rupture: C.—Follicle cavity; G.—Granulosa layer; Th. I.—Theca interna; Th. Ex.—Theca externa.

Fig. 10—Early corpus luteum, beginning vascularization: C.—Cavity filled with blood clot; G. Lut.—Granulosa lutein layer already invaded by capillaries; Th. I.—Theca interna; Th. Ex.—Theca externa.

Fig. 11.—Well developed corpus luteum: C′.—Central cavity filled with organized connective tissue; Lut.—Lutein layers resembling adrenal cortex; Th. I.—Theca interna; Th. Ex.—Theca externa.

Corpus albicans. The regression of the secreting elements becomes more and more apparent and their replacement by connective tissue is evident. For a long time, sometimes for months, the crenated area, previously occupied by the lutein cells, is still recognizable as a homogeneous acidophil (eosin) staining band in the stroma of the ovary. (See Fig. 15).

The corpus luteum of pregnancy which, as was mentioned, is larger and more developed than the corpus luteum of the infertile cycle (incorrectly termed of menstruation) because of its longer life history, is able to form very regular, well defined connective tissue septa through which larger and thick walled vessels penetrate into the interior of the yellow body. The theca interna cells are found in these septa. After the fourth month of pregnancy, considerable quantities of colloid are found in the lutein cells (Miller, 339). Calcium deposits are likewise of frequent occurrence in the corpus luteum of pregnancy.

Persistent Corpus Luteum

As Tandler (436) pointed out, in the cow a corpus luteum may persist and apparently function in the absence of pregnancy. This, as will be shown later, (page 54) produces a prolonged stage of estrus or an inhibition of a new estrous cycle. Reynolds (388) has described the same condition in the human without, however, convincing me of the value of the criteria he employed in recognizing a persistence of the yellow body.

Robert Meyer (336) has described *accessory lutein formation* in atretic follicles. In addition, theca lutein proliferation in atretic follicles during normal pregnancy, accentuated especially in the presence of hydatid mole and chorion-epithelioma, has been repeatedly observed. The resultant ovarian cystic enlargements are called lutein cystic ovaries (Findley, 122). Such ovaries may form pseudo-tumors which often regress after the uterus is emptied (Fraenkel, 131). These findings which previously were quite inexplicable, will again be referred to in connection with the experimental luteinization produced by means of anterior lobe implantations (Evans and Burr, 109) or anterior lobe extracts (Zondek and Aschheim, 486), (See page 14).

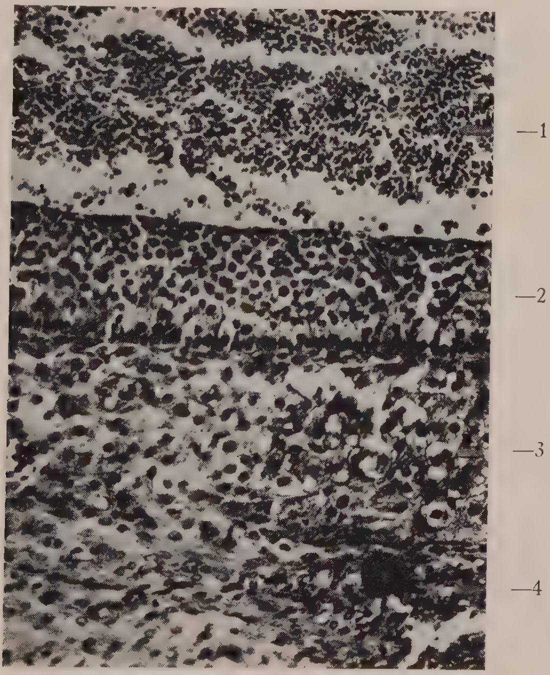


FIG. 12. PHOTOMICROGRAPH OF ATRETIC FOLLICLE.

1. Follicular cavity filled with debris. 2. Granulosa layer. 3. Theca interna. 4. Theca externa.

(Figs. 12–16 inclusive are taken from Frank, Gynecological and Obstetrical Pathology, Appleton & Co., 1922.)

ATRESIA

As was previously mentioned, of the many thousands of ova contained in the gonads at birth, at most 360 to 420 fully mature and rupture. Thousands of ova, especially after maturity is reached, grow, mature to a certain degree, and then from causes not entirely clear, regress at any stage in their development, and finally degenerate. This involutionary process is recognizable by the destruction of the marginal differentiations of the theca interna and externa.

In pregnancy the process of atresia seems accelerated, and is accompanied by a marked lutein change in the theca interna cells which are often then called theca lutein cells (Wallart, 465 and Seitz, 413). The resultant ovarian changes have led Wallart to call this the human interstitial gland.

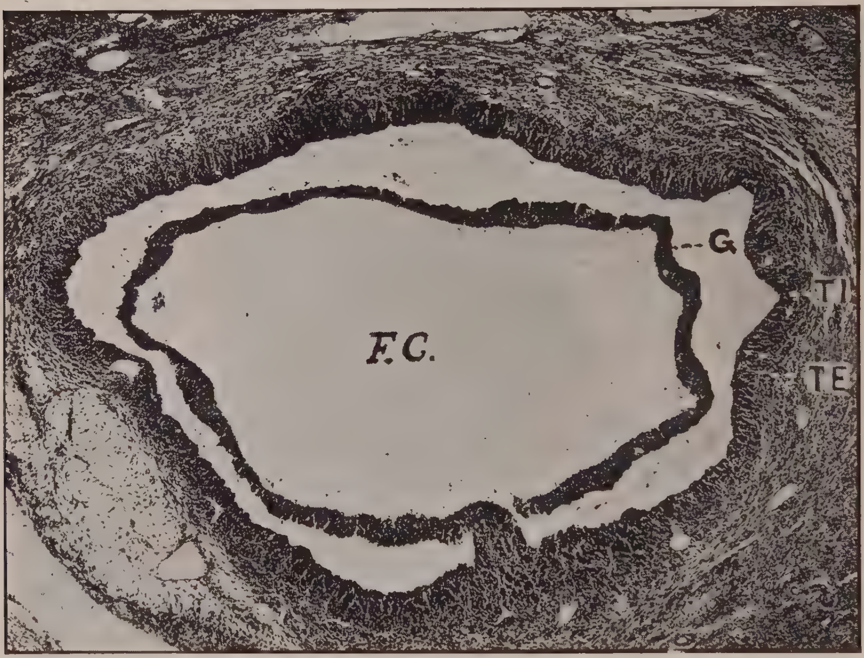


Fig. 13. Cystic Atresia. Early Stage. (Photomicrograph.)

This transverse section across atretic follicle shows the granulosa layer (G) separated from theca interna (T. I.) which in turn abuts against the theca externa (T. E.). The follicle cavity is still filled with fluid (F. C.).

The final stage of atresia produces a corpus albicans not distinguishable from that resulting from regression of a corpus luteum. Moreover, large atretic follicles are often indistinguishable to the naked eye from ripening follicles.

I am inclined to believe that much of the female sex hormone generated throughout the cycle is produced by the many growing follicles which early become atretic. This source of the hormone would account for its production throughout the age of prepuberty as well as for some of the production which is noted during the cycle.

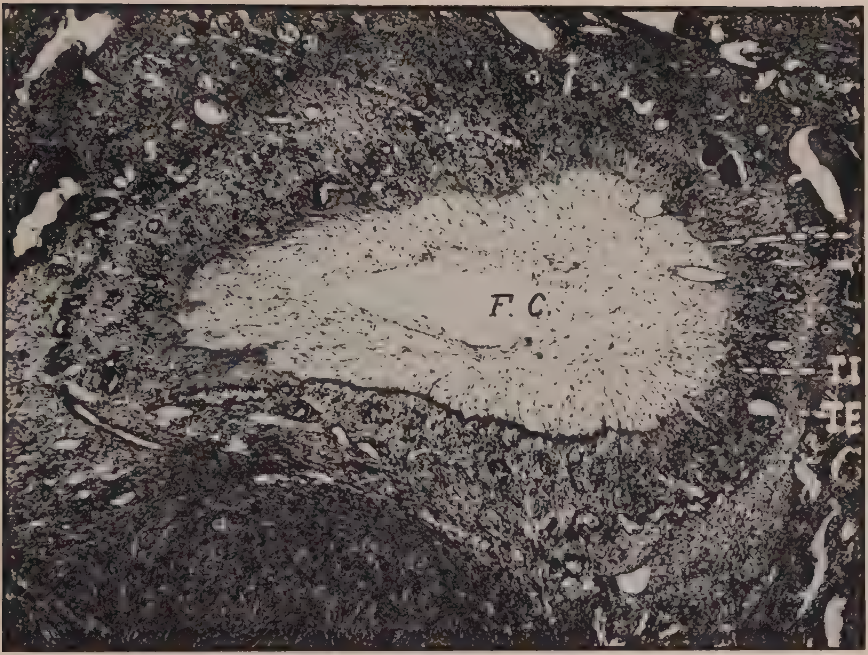


Fig. 14. More Advanced Atresia. (Photomicrograph.)
The follicle cavity (F. C.) is almost obliterated by the ingrowth of connective tissue. The granulosa layer has almost disintegrated. The theca interna is still distinguishable.

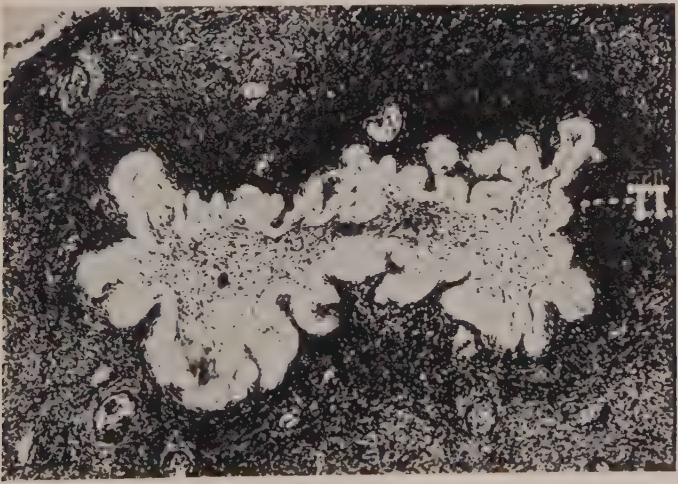


Fig. 15. Advanced Atresia. Corpus Atreticum. (Photomicrograph.)
The follicle cavity is obliterated. A hyalin band still demarcates the remains of the collapsed and distorted atretic follicle from the invading ovarian stroma.

Interstitial Gland

The interstitial gland has been called the puberty gland by Steinach (429). This appears to be a misnomer, especially if further investigation proves the contention of Smith and Zondek that puberty is initiated by the anterior pituitary (see page 14). The interstitial gland, if present, consists of scattered epithelial or epithelial-like agglomeration of cells or of large cell groups throughout the cortex of the ovaries of certain animals. According to the investigations of Fraenkel (128–9) the interstitial gland develops in only 50 per cent of the eighty species investigated. Variations

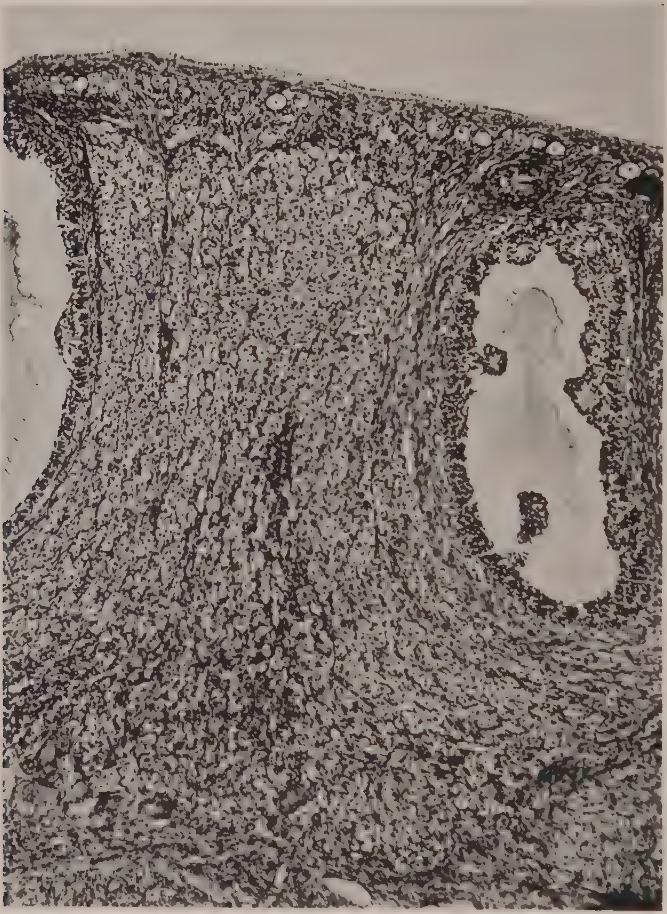


Fig. 16. Interstitial Gland of a Rabbit. (Photomicrograph.)

Between two large follicles is seen the ovarian stroma composed mainly of interstitial cells. These cells are laid down in columns and resemble lutein cells.

in the development of the gland to a greater or minor degree in the same species depend on the season of the year, being most massive after the onset of puberty and during the sex season. Bouin and Ancel (53) attempted to divide species into those having no interstitial gland, in which ovulation was supposed to be spontaneous, and those in which an interstitial gland was present, in which ovulation was supposed to be dependent upon coitus. Their contention has not stood the test of time. The inconstance of this gland is well shown by the research of Mey (334) who found it well developed only from the last month of fetal life in calves up to the second week of extra-uterine existence. From then on, rapid involution took place.

Limon (286) traced the origin of the interstitial gland from connective tissue structures. Lately Wilkerson again claims its origin from the theca interna (470). Lane-Claypon (261) believes that the interstitial gland is formed from primordial follicles, i.e., of epithelial origin. Wallart (465), as previously mentioned, considers the luteinized atretric follicles found in the human being during pregnancy, identical with the interstitial gland of animals. No function can as yet, with any degree of certitude, be assigned to these inconstant structures.

Tubular Tract

(*Uterus, Fallopian Tubes, Vagina*)

Uterus. In analyzing the sex cycle and in studying the sex organs, it should always be kept in mind that the sole *raison d'être* for the sex organs is to subserve the function of reproduction of the species. Each sex cycle is directed toward this sole objective. In the most prolific human females approximately 1.5 per cent of cycles attain this aim.

Previously we have considered the sex cycle in the ovary. Now, we shall study the sex cycle in the uterus. For some time the intimate co-relation between the activating ovarian and the responding (passive) uterine cycle have been well established and recognized. The work of many investigators has finally clarified the issue. Among those deserving special mention are Sobotta (425), Leo Loeb (301), Robert Meyer (335–7), Carl Ruge, Jr. (397), Robert Schröder (407, 409), J. Willoughby Miller (339), and others too numerous to refer to.

In order to comprehend fully the possibilities, as well as to understand the phenomena at issue, it is necessary to give a short outline of the physiology of the cycle which will be discussed in greater detail elsewhere.

The Cycle in Mammals Below the Primates.

The sex season is that period during which the sex organs periodically become active. A *monestrous* cycle is one in which only one cycle occurs during each sex season. A *polyestrous* cycle is one in which repeated cycles occur during a sex season, usually under normal circumstances, until pregnancy supervenes.

According to the nomenclature of Heape (214–5) the sexual cycle of the mammals may be divided into the following stages—*anestrus*, the quiescent or resting stage; *proestrus*, "coming on in heat," during which turgescence of the uterus, vagina, and vulva increases, together with endometrial changes (glandular hyperplasia, epithelial and stromal mitosis, secretory phenomena); *estrus*, the period of heat and period of desire, during which the endometrial changes are well marked and in which in certain animals, as in the rat, the uterus may be filled with fluid. The final stage consists in *pregnancy*, if impregnation has taken place, in which the changes incident to gravidity occur in the uterus, vagina, and vulva. *Pseudo-pregnancy*, a period of prolonged hyperplasia, well marked in certain forms, as in the bitch, supervenes if pregnancy does not take place. This phase is short and hardly recognizable in some other species. The cycle is repeated by a return to anestrus.

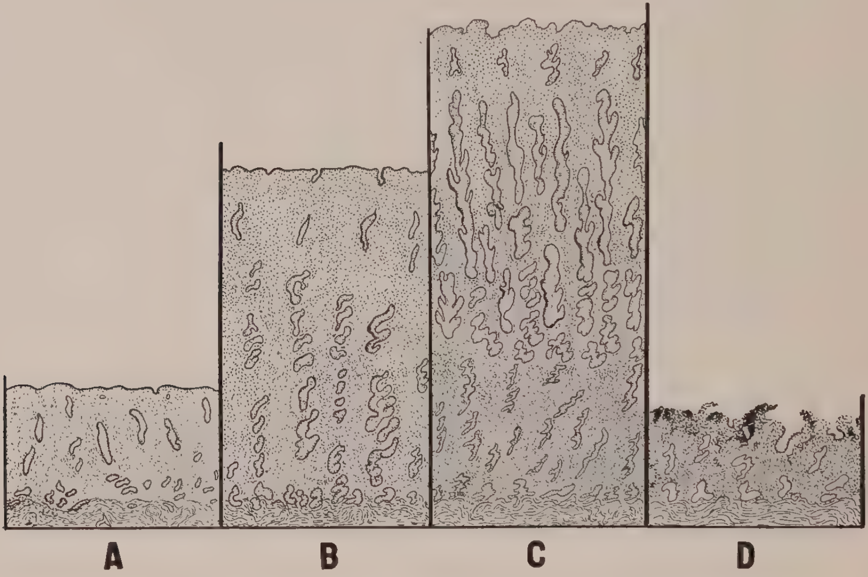


Fig. 17. Diagram of Human Uterine Mucosa During Menstrual Cycle.

A. Resting stage. The mucous membrane is thin, the glands far apart and simple.

B. Interval. The mucous membrane is greatly thickened. The glands have assumed a typical corkscrew shape and are more closely packed. There is greater vascularity.

C. Pregravid stage. (Premenstrual.) Still greater thickening of the mucosa is noticed. In the upper two-thirds the glands have multiplied, become tortuous and close packed. The superficial third is known as the compact layer, the middle third as the spongy layer (the two together as the functional layers). The almost unchanged deepest layer of the endometrium is called the basalis.

D. Menstruating mucosa showing desquamation of the functional layer with torn and hemorrhagic basalis remaining.

The Cycle in Primates; Especially in Man.

Certain special changes in the cycle are noted in the primate series. The most striking of these is the occurrence of menstruation if pregnancy does not take place. These changes were studied in the human being as early as 1873 by Kundrath and Engelmann (259) and their work was rediscovered and amplified by Hitschmann and Adler (226). Heape, in 1893, (213) studied the cycle in *semnopithecus entellus*, a variety of monkey.

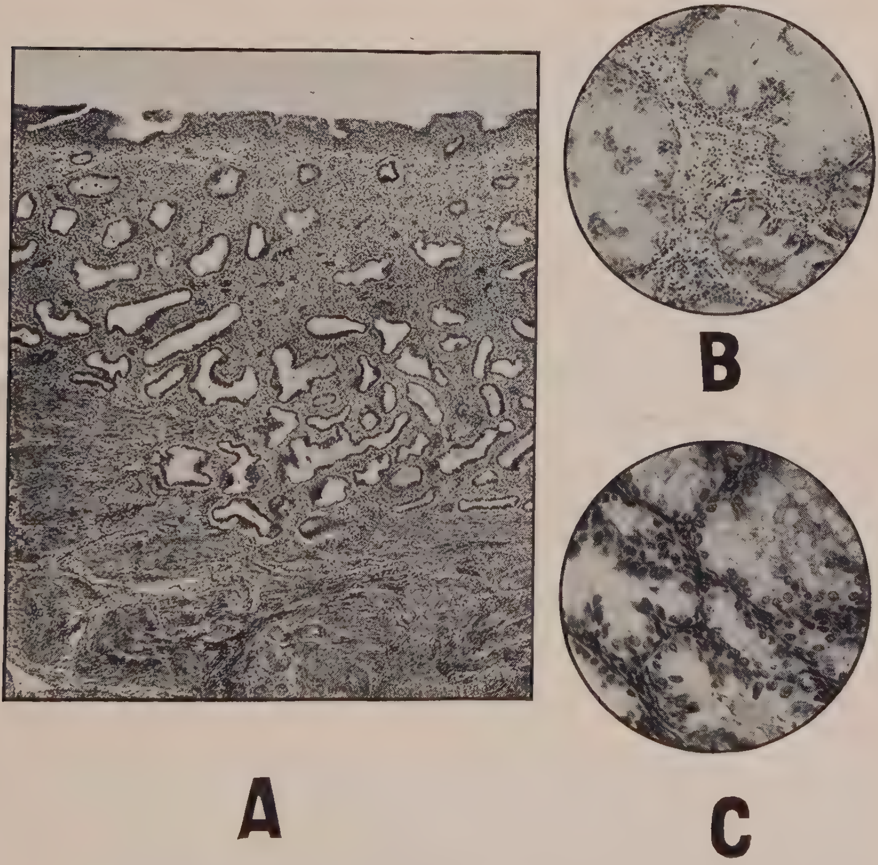


Fig. 18. Human Endometrium. (Photomicrograph.)

A. Resting mucosa showing simple glands, an irregular outline demarcating endometrium from musculature. (Low power.)

B. Endometrial glands, interval stage. Convolution of gland outlines, beginning of feathering of epithelium is shown. (High power.)

C. Pregravid stage, showing secretion of epithelium and narrow bridges of stroma in the "spongy" layer. (High power.)

The following subdivision of the cycle can be made. *Rest*—the uterus being small, pale, with very thin mucosa, containing straight, simple glands, with wide interglandular spaces. *Interval*—during this stage a slow growth of the mucosa takes place, without secretion. *Pregravid* (pre-menstrual)—this stage is marked by turgescence, hyperplasia of the glands, showing itself by tortuosity of the gland contour and feathering of its epithelium. Intraglandular secretion increases. The stroma undergoes well marked decidual changes. *Pregnancy*—with nidation of the ovum, its further development, and the uterine and other changes incident to pregnancy. Or *Menstruation.*

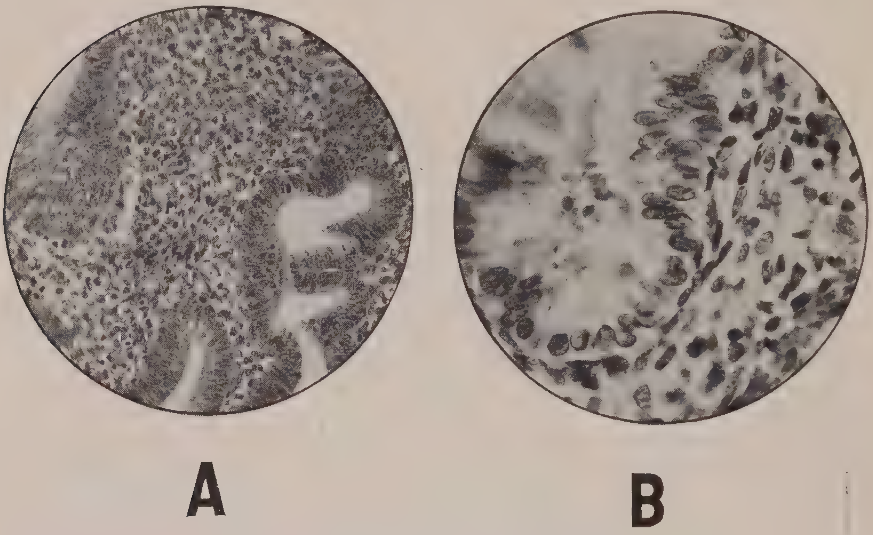


Fig. 19. Human Endometrial Glands.

A. High power, beginning interval. Single layer of epithelium with well defined margin toward lumen. Connective tissue type of inter-glandular stroma.

B. Pregravid stage, showing feathering of epithelium and secretion. Decidual change of stroma.

The mechanism of menstruation apparently can be one of three varieties:

(1) A rapid and complete necrosis of the so-called functional layers which includes the compact and spongy layer. The muscle is then only covered by the remaining basal layer containing the fundi of glands from which repair later takes place. These changes were described by Schröder (407) and Lindner (287), who both found fragments of the endometrium in the menstrual discharges.

(2) An entire absence of endometrial exfoliation, the hemorrhages taking place though the small hematomata which lift off the surface epithelium or by means of diapedesis, Gebhard (174). Regression then occurs by gradual involution of the mucosa without necrosis.

Corner (74), (495) and also Hartman (208a) have described menstruation from an inactive almost resting mucosa in monkeys without any exfoliation.

The bleeding from the vagina and uterus noted in cows, bitches and mares during the height of estrus neither coincides in time nor is in any way analagous to the menstrual bleeding of the human female.

CORRELATION OF OVARIAN AND UTERINE CYCLES

Before we were able to correlate the ovarian and uterine cycle by reproducing the ovarian stimuli in physiological experiment, the intensive investigation of many zoologists and clinicians had already given us an excellent *aperçu* of the parallelism of these phenomena.

Historical

Of interest is the work of Leopold and Miranoff (280) who denied any correlation. Fraenkel (127) on the other hand at first ascribed the production of menstruation to the corpus luteum, but later abandoned this position. Robert Meyer (335) started out with the impression that there was no such correlation, but later on changed his point of view and has become one of the fervent sponsors of the correlative theory. All of this work was mainly based on human material. The difficulty of obtaining normal human specimens is self-evident.

Time of Ovulation

Based on their studies of human material, Meyer (337) and Ruge (397), especially on observation of recently ruptured follicles, concluded that ovulation occurred between the first and fourteenth day after the onset of the preceding menstruation. Schröder (407) agreed with their findings. Miller (339) as well as Fraenkel (130) placed the date of ovulation about the eighteenth day after the onset of the last menses. Recently the actual time of ovulation in primates has been demonstrated by finding the ova in the tube.

The Time of Ovulation in Different Species

(a) Spontaneous ovulation usually occurs with the onset of, or during the time of estrus. This is so in the mouse, rat, guinea pig, hog, bitch, sow, cow, and mare.

(b) Ovulation post coitu occurs in the rabbit, Regaud et Dubreuil (384), Heape (215); ferret, Robinson (392); and cat, Longley (321).

(c) A pseudo-dissociation between estrus and ovulation has been noted in the bat. Coitus takes place in September. A maturing follicle persists throughout the winter until the month of April and May. Ovulation does not occur until then. Impregnation results through the many spermatozoa stored in the uterus throughout the entire winter (Eimer, 104; Van der Stricht, 449; Courrier, 76).

(d) In the primate ovulation has been definitely determined at the following times of the cycle. Corner (74) in the rhesus found ova in the tube on the fourteenth day and in the uterus on the seventeenth day after the beginning of the last menstruation. Hartman (208) likewise in the rhesus found a fresh, ruptured follicle on the eleventh day. Allen and Pratt (11) found human ova in the tube on the tenth and fourteenth day.

The question as to whether pregnancy and lactation inhibit ovulation is taken up on p. 54.

Does and Can Menstruation Occur Without Ovulation?

Heape (213) in 1895 examined 42 semnopithecus entellus monkeys with menstruating uteri, in not one of which either a ruptured follicle or a recent corpus luteum could be seen. Corner (74) has made the same observation in a number of macacus rhesus monkeys although in some ovulation had taken place. Schröder (408) as well as Geist (176) have shown irregular bleeding to occur in human beings although no corpus luteum, which would document a preceding ovulation, could be found.

In my opinion atresia can under certain conditions supply a sufficient amount of female sex hormone to bring the uterus of the primate into a full pregravid, or, as it is also called, premenstrual condition. If then, for one reason or another, the hormonal stimulus is abruptly withdrawn, the activated uterine mucosa can and will respond by the phenomena of menstruation. This is fully proved by the striking experiments of Pratt and Allen (378) who injected female sex hormone into castrated monkeys (also Allen, 5). The abrupt withdrawal (the cessation of injections) was followed by a typical menstrual flow in seven of the ten monkeys used.

The Causes of Ovulation

Increase in ovarian tension was suggested by both Pflüger (375) and Strassmann (434) as causing the rupture of the follicle. The congestion due to coitus may have its effect in animals such as the rabbit, ferret and cat in which follicle rupture rarely occurs except after conjugation.

Independence from nervous influences was demonstrated by Knauer (253) who found ovulation in transplanted ovaries of rabbits, and Halban (199) of monkeys. Goltz (188) noted regular menstruation and impregnation in a woman afflicted with transverse myelitis.

Enzymatic weakening of the follicular capsule was suggested by Schochet (404) followed by rupture.

CYCLE IN THE TUBES AND VAGINA

Tubal Cycle

Geist (175) showed that in the human Fallopian tube certain changes in the epithelium occurred during the cycle. They are not well marked nor apparently of great importance in the human.

Courrier and Gerlinger (82) describe a marked secretory reaction in the tube of the bitch while the ova are traversing it, and Courrier (79), in the rabbit as the follicle is ripening. The change in rate of spontaneous contraction in the fallopian tube incident to the cycle is discussed on page 95.

> *The rate of passage of the mammalian ovum* through the Fallopian tube varies in different species. Dorothy Anderson (17) has studied the rate in the sow. The following data are given by Anderson for the traversing of ova to the uterus in different species.

In rodents	4 species	about 3 days
In ungulates	2 species	about 3 days
In carnivora	3 species	about 5–10 days
In marsupials	2 species	about 1 day
In bats	4 species	more than several weeks.

Vaginal Cycle

As early as 1892 and 1893, Morau (342) and Lataste (276) pointed out distinct cyclical changes in the vaginal walls of rodents. This was further studied by Retterer (386). More recently Stockard and Papanicolaou (433) have studied the vaginal cycle in the guinea pig. They showed that the vagina opens at puberty and that the content of the vaginal secretion changes characteristically throughout the cycle, so that from its character it is possible to predict the condition of the inaccessible uterus and ovaries. They further pointed out that these cyclical changes were replaced by a static vaginal content throughout pregnancy. Long and Evans (320) described these changes in the vaginal smear of the rat. Allen and Doisy (6) utilized these findings to elaborate a practical test for the recognition and assay of the female sex hormone, (see page 88). Kelly and Papanicolaou (249) more recently have shown that in the guinea pig a temporary agglutination of the vulvar lips takes place, the vulvar outlet being completely shut off for 85 per cent of the cycle and open only during estrus.

Although some cyclical changes can be noted in the vagina of many species, these variations are not as striking as in the rodents.

Wilson (472) in the sow found that the vaginal smears are not reliable in determining the actual time of the cycle.

Jesse King (251) in the human female likewise found the smears inexact. Papanicolaou (355) has claimed their diagnostic value in women, but as yet he stands alone in successfully interpreting them.

Adler, 1928 (1) by means of excisions of vaginal mucosa in the human, determined the presence of a "functional" layer in the vagina. The method is of no practical importance.

Changes in the vaginal bacterial flora ascribed to the cyclical variation of the glycogen contents of the vaginal epithelium were described by Lehmann (277) in 1921. At the time of the approach of menstruation, and especially during pregnancy, the glycogen is increased.

OTHER CYCLICAL PHENOMENA

The Breast Cycle

Lane-Claypon and Starling (262) mistakenly ascribed the changes occurring in the breasts to stimuli emanating from the fetus. The error in their experimentation was pointed out by Frank and Unger (166) who agreed with Ancel and Bouin (13) in ascribing the cyclical breast phenomena to the effect of the corpus luteum. Two years later (1913) Aschner (27), Fellner (114), Schickele (401), by means of placental and corpus luteum extracts, produced activation of the breast experimentally.

In animals, and the rabbit for morphological reasons lends itself best to this type of experimentation, it is found that the small, barely visible anlage of the breast remains quiescent until the onset of puberty. Thereupon, under the influence of the increasing concentration of the female sex hormone in the blood, a tree-like extension of the mammary ducts takes place. If this stimulus is sufficiently prolonged, alveoli are found resembling the leaves of a tree (see Fig. 24, p. 59). When the stimulus is withdrawn at the end of estrus and involution takes place, regression of the alveoli but not of the duct structures results. These changes appear in all species, but are more difficult to demonstrate in certain types, as for example in the rat. For details see Frank and Unger (166). In the human female, Rosenburg (396) has studied the process with care. This monthly excitation of the breast structures may account for the frequency of the occurrence of carcinoma in the female, in contradistinction to its rarity in the male breast.

Changes in the General Organism

Lack of space prevents a discussion of the cyclical changes produced in many of the organ systems. The leucocytes, the blood platelets, the coagulation time, the bleeding time, and the temperature curve, the basal metabolism have all been studied in the human female in this connection, but the results have been inconclusive or contradictory. A difference in the reaction to certain drugs, as for example the response to atropine, pilocarpine and adrenalin has been shown to exist (338). The difference in the blood-level of the female sex hormone throughout the cycle will be discussed on page 189. The fluctuation in the nervous response and psychological condition of the female will be referred to also on page 209.

Changes in the Symphysis Pubis

Some very special mechanisms which occur in certain species must be referred to later and are therefore mentioned in this connection. Hisaw (224) found that the symphysis pubis of the pocket gopher underwent softening and absorption during the height of the cycle, a mechanism evidently of use in connection with pregnancy and labor. Since then he has applied a somewhat similar mechanism which occurs in the symphysis of the virgin guinea pig, for testing out the effect of certain corpus luteum extracts (Todd, 442) (see page 99).

The Uterus as a Gland of Internal Secretion.

Bond (51), as well as Blair Bell (38), have mentioned the uterus as possibly a gland of internal secretion.

Ancel and Bouin (14) described a "glande myometrale" appearing in the endometrium at certain phases of the cycle.

Loeb (301), as well as others, have noted that after hysterectomy, increased cystic atresia may occur in the ovarian follicles. This appears to me to be a partly mechanical, partly circulatory effect due to the impairment of the utero-ovarian vascular anastomosis.

Zimmermann, 1923 (479) interpreted the increase in size of the rabbit's ovaries found 10–12 months after hysterectomy as due to lack of function. According to him, implantation of endometrium relieves this.

We (Frank and Goldberger) (page 256) have shown that in the human female after hysterectomy, the hormonal blood-cycle may continue unchanged.

Gentili (181) has endowed the decidua with an endocrine function but has offered no evidence in substantiation.

PREGNANCY

The Ovum

To the ovum throughout its course through the tube and its presence in the uterus has been ascribed a certain guardian or trophic influence by Robert Meyer (337).

The following three quotations sufficiently state his views.

> ". . . . throughout, from the first development of the primordial follicles until the death of the ovum, the development of the follicle depends on the fate of the ovum." (Page 16.)
>
> ". . . . to the lutein producing influence of the ovum is added the lutein persisting influence of the fecundated ovum." (Page 18.)
>
> "This is both within and outside of the ovary. The living ovum influences the development, the maintenance and accumulation of lipochromes (lutein) and also the inhibition of other ova."

Allen and Doisy (9), apparently unaware of Meyer's views, have voiced the same hypothesis.

I have never been fully convinced of this functional effect of the ovum after its extrusion from the follicle and before its nidation in the uterine mucosa. Until quite recently such a possibility could not be denied. There is no question that the ovum, after nidation has taken place (that is imbedding within the uterine mucous membrane of the fertilized ovum) exerts a hormonal influence, but this process according to Grosser (193) requires from 8 to 10 days in the human.

Recently Westman (469) has excised the tubes of animals harboring both impregnated and unimpregnated ova, without in any way affecting the development or persistence of the corpus luteum. This appears to disprove Robert Meyer's views, as well as Allen and Doisy's independently expressed view of the dominant influence of the unimbedded ovum.

The Placenta

Furthermore, as soon as the first chorion ectoderm is formed and thereafter when the chorion epithelium develops, this precursor of the placenta, as well as the later placenta, act as a gland of internal secretion, producing a large amount of female sex hormone. This influence was first suggested by Halban (200) in 1905 and demonstrated by Iscovesco (231) by means of placental extracts in 1912.

This is no place to enter into the details of nidation but it may be well for the sake of clearness to emphasize the fact that the placenta, when definitely formed, consists of two portions, a maternal decidual portion, which develops differently in each species, and a fetal portion, the chorion ectoderm, which is an active organ of internal secretion.

The placental attachment in different species varies between a loose relation, in which the maternal and fetal surfaces are separated by cellular debris, through species in which the placental villi project into hardly changed uterine glands, to the higher species in which maternal and fetal surfaces are intimately fused in such a fashion that the blood stream of the mother and that of the offspring are separated merely by a living membrane consisting of maternal endothelium and fetal chorion ectoderm (syncytium and Langhans-cells).

In the viviparous fish, torpedo occellata, the maternal and fetal parts are completely separated by a layer of fluid. In the pig, with diffuse placenta, the maternal uterine epithelium persists. In herbivora the villi dip into the uterine glands but fuse only at the tip. In rodents the maternal surface epithelium disappears at the placental site. For further details see Grosser (193).

The Blood

In pregnancy, to anticipate what follows later, there is a huge increase in the circulating amount of female sex hormone in the blood (see page 194) as well as of the anterior pituitary secretion (see page 196).

Local Changes

In consequence of these increased hormonal stimuli and perhaps also as a result of local effects produced by the growing ovum, tremendous changes occur in the uterus, vagina, and breasts. These changes are essential for the safe harboring and fostering of the growing young. They are also necessary to permit labor to take place. The breast changes are essential for the early nutrition of the newborn.

Systemic Effects

In addition to these readily understood changes coincident with pregnancy, other less readily explainable phenomena occur. Among these are the so-called osteophites within the skull, described by Rokitansky (394) and other bone and connective tissue hyperplasia resulting from anterior pituitary stimulation (see Hofbauer, 227).

Adrenal hyperactivity is said to produce pigmentary anomalies. The pancreas function is affected early in pregnancy as evidenced by a lower renal sugar threshold. The thyroid is usually stimulated. These associated pregnancy changes were dealt with by me in an article in 1917 (140).

Anatomically recognizable changes can be determined in the anterior lobe of the pituitary. They consist of increase in size of the adeno-pituitary, increase in number of the colorless, chromophobe (Hauptzellen) and basophile elements (pregnancy cells) (Erdheim and Stumme, 107). No similarly striking changes can be noted in the other glands of internal secretion except in the ovary. Here persistence of the corpus luteum (of pregnancy) has already been referred to, as well as the cessation of ovulation and the increase of atresia with consequent increase in theca-lutein tissue.

This concludes a most brief and summary survey of the anatomical changes noted during the cycle. It is essential to describe them, in order that reference to them in subsequent chapters may be understood. It is now my purpose to turn to the experiments performed between 1896 and 1923, which served to supply a farther insight into the physiological processes which produce the changes referred to.

CHAPTER III

PHYSIOLOGICAL EXPERIMENTS

CASTRATION—TRANSPLANTATION—IMPLANTATION—ABLATION OF THE OVARY

THE CORPUS LUTEUM

NEITHER the hormone theory itself, nor its application to the female generative tract was formulated at one time and by one sponsor. In 1848 Berthold (41) performed implantation experiments with the gonads of cockerels. This important achievement aroused little attention and was soon forgotten. On the other hand, the spectacular but false experiments of Brown-Séquard (62) with testicular extract, because they appealed to the dramatic instincts and credulity of the crowd, have reechoed through succeeding decades and have inspired research in every branch of endocrinology.

This chapter is designed to portray some of the experimental and theoretical work appearing between 1896–1923. From 1923 to the present a striking speeding-up of the work in female sex research has taken place requiring separate consideration.

CASTRATION

Castration was known in prehistoric times. The agriculturist practiced it on the males of cattle and horses to make them more tractable. The oriental monarch guarded his harem with eunuchs. Castration of the female was a less common and widespread practice because of its greater dangers and greater technical difficulties. The effects of castration differ greatly depending upon the time of life at which performed.

In Infancy

No reliable record of a case of early castration and survival to adult age exists in the human. If young female animals are castrated, the tubular sex tract (uterus, vagina, vulva) as well as breasts (Foges, 126) remain undeveloped and even atrophy. The secondary sex characters remain in abeyance. The epiphyses ossify late with resultant long extremities. In other words, a neuter type results.

The hypophysis in castrates is invariably enlarged (Erdheim and Stumme, 107). In animals libido does not develop. The basal metabolism is but slightly affected. We will refer to this later.

The special effects produced by castration are fully discussed in the monograph of Tandler and Grosz (437).

In Adults

Castration practiced on adults produces rapid involution of the tubular tract including atrophy of the vulva. The breasts likewise involute. The labile secondary sex characters may be affected. This includes falling out of the hair, occasionally, premature graying. Fat distribution varies. In thin women the spareness is often accentuated, while those inclined to obesity, unless submitted to a careful diet, show undue increase in fat accumulation. In the human female, libido is rarely, if ever, influenced. Needless to say the cycle abruptly ceases, no further menstrual periods being noted.

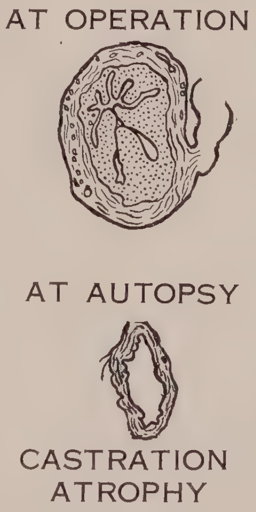


Fig. 20. Cross Section of Rabbit's Uterus.

Outline drawings from cross sections projected on paper with Edinger projection apparatus. Above, cross-section of mature rabbits' uterus at time of castration. Below Castration atrophy as noted 10 days later.

The occasional reports of *menstruatio sine ovarii* (see Gellhorn, 180), are due to the fact that in inflammatory disease complete removal of the ovaries may prove difficult or impossible. Such undetected ovarian fragments may later cause menstruation. Regeneration of ovarian tissue from the bursa ovarica of castrated mice occurs with frequency. (Davenport, 88).

Quite contradictory reports on the basal metabolism have been published. Loewy and Richter (319) claimed that the basal metabolism of castrated women and dogs was reduced at least 10 to 15 per cent. The accuracy of their observations is somewhat beclouded by their report that after oral administration of desiccated ovarian substance, the basal metabolism returned to normal. These ovarian substances contain such minute quantities (if any) of active substance, and oral administration is so ineffective, that these findings cannot be accepted as conclusive (see page 265). McCrudden (332) who studied the basal metabolism as well as the calcium, phosphorus, nitrogen and other metabolic factors in detail, found that castration of both male and female did not change the metabolic rate.

Murlin and Bailey (345) studied most carefully the basal metabolism of dogs and found a slight reduction in oxygen consumption after castration. Geist *et alia* (177), in 50 women examined for considerable periods after castration, found such a small reduction in basal metabolism that it appeared of little clinical importance.

Slonaker (417) found an increase in activity displayed by albino rats during estrus. This increase in activity was entirely eliminated by castration.

Unilateral castration has no apparent effect on the sex cycle (Arai, 18). The same number of follicles ripen as if both ovaries were present. The same number of corpora lutea develop and the same number of young are born. Hence as the quantity of ovary is reduced by half, there must be a more rapid consumption of follicles when compared to the total amount present in reserve (Asdell, 29).

Fragments of ovary left *in situ* may function normally at least for a limited period. Bucura (63) in 1907 claimed that a minute fragment consisting of nothing but a single follicle which matured, was able to produce a full estrous cycle in a rabbit.

TRANSPLANTATION

Intermediate between true transplants and other technics is the so-called *ectropionization* practiced by Villemin (455) in which he transposed the ovary, without interfering with its pedicle, from within the peritoneal cavity into the layers of the abdominal wall. This transposition produced no change in the cyclical phenomena.

Transplantation may be practiced upon the host (auto-transplants), upon another animal of the same species (homo-transplants), to an animal of different species (hetero-transplants).

Knauer, in 1895, (253) performed auto- and homo-transplantation upon rabbits. In 1897, Grigorieff (191) similarly homo-transplanted ovaries of rabbits, the transplants being subsequently followed by pregnancy in the new host. In 1900, Halban (199), by transplanting the ovaries of other monkeys into castrate monkeys, was able to restore the menstrual function in the operated castrate.

Many, and on the whole, little convincing transplantations have been performed on human beings. The literature can be found in Martin (327-8) and Tuffier (447). One of the reasons that all such reports should be accepted with reserve is the fact that when castration is performed on the human female in the presence of severe inflammatory pelvic lesions, it is sometimes impossible even for an experienced operator employing the best technic, to avoid leaving small fragments of ovarian tissue in the operative field. This happening accounts for such reports as that of Gellhorn (180) menstruation without ovaries, or the case reported by Hirst in the discussion of my paper in Philadelphia (Frank, 151) in which after a supposed complete castration, pregnancy developed.

Technic

It is universally accepted that auto-transplants succeed in a much greater percentage than homo-transplants. The immediate insertion of thin slices of ovarian tissue into a properly prepared and situated wound bed favors success. It is more than doubtful that permanent success can be obtained with hetero-transplants even in such closely related species as the mouse and rat. Little if any importance seems to attach to similarity of blood-grouping between donor and recipient in human species (Pettinari, 373). Dudley (102) devised a technic which may still promise success after complete removal of the tubes, namely transplantation of the ovaries into the horn of the uterus in such a fashion that ovulation takes place directly into the uterine cavity.

Transplantation of Ova

Biedl, Peters, and Hoffstätter (45) in spite of almost insurmountable difficulties, in one instance of a large series, succeeded in transplanting fertilized ova from one rabbit into another, the pregnancy going to term in the foster mother. In several instances they were able to produce nidation which, however, terminated in abortion.

The few successes in such a large series of carefully conducted experiments led me to doubt the "primacy of the ovum" which Robert Meyer (337) as well as Allen and Doisy (9) maintain. If this were the case the newly implanted ovum should be able, during its course through the Fallopian tube, to exert its influence on the ovary and thus assure the hormonal response and activation of the uterus. For further discussion of this fascinating theme see page 41.

Transplantation of Other Sex Organs

Loeb (302), as we shall mention later in more detail, was able to transplant fragments of a uterus containing deciduomata in other portions of the same host, with slightly continued growth of these artificial tumors. Ribbert (389) successfully transplanted the breast of a guinea pig into the ear of the same animal. This breast secreted in its new location when pregnancy ensued. Frank and Unger (166) were able to grow portions of placenta and portions of fetuses in the peritoneal cavities of rats. Grafts of fetuses in the anterior chamber of the eye likewise continued to grow for a considerable period of time. Frank (139) transplanted finely subdivided uterine tissue, both auto- and homo-transplants, into castrate rats. Injections of the female sex hormone produced a great increase in percentage of takes as well as increased rapidity of growth. These transplants likewise involuted after a certain time had elapsed. Schochet (405) was able to transplant uterine endometrium (auto-transplants) into the anterior chamber of rats and guinea pigs where the cyclical phenomena could be watched. (See also Markee, 323).

In Vitro

Frank (135) placed *in vitro* transplants of normal uterine tissues in Carrel-Burroughs medium, one series in the presence of, the other without fresh corpus luteum particles. No difficulty was found in getting the connective tissue stroma to grow. Epithelial growth was occasionally obtained. Similar experiments were performed in an attempt to cultivate and stimulate the growth of artificial deciduomata. The connective tissue growth that was obtained differed in no way from that seen when normal uterine or ovarian tissue or even rat sarcoma was employed. The deciduoma tissue showed less vitality and less proliferation than normal tissues.

Confirmation of my work is offered by Courrier (80) in 1925, who used *in vitro* cultures of the vaginal epithelium of the castrated female rabbit. He attempted to stimulate with "folliculin" with negative result.

Implantation

Implantation is a method which takes advantage of osmotic processes, by means of which substances contained in the cells of the implanted fragments enter the circulation of the host. It is another method of parenteral injection which permits the utilization of delicate and unstable substances which lose their potency during extraction.

Philip Smith (421) succeeded in obtaining results with anterior lobe implants used extremely fresh, only one or two seconds elapsing between the time of the removal from the host and the implantation into the recipient. These experiments have been referred to (page 14).

Zondek and Aschheim (483) employed the method of implantation in order to test the activity of various components of the ovary. Minute fragments of granulosa, of theca interna, of corpus luteum, etc. were transplanted in the thigh musculature of castrated mice and by this simple technic the contents in female sex hormone of these tissues could be evaluated by the vaginal spread reaction (see page 122).

The reaction obtained by implantation produces an early effect if the result is positive, due to the exosmosis of active substances in the host. The results show themselves in at most two to three days and are of very transient duration. This distinguishes implants from transplants in which the real effect is only noticed after full vascularization of the transplanted fragments, the noticeable result then occurring in from two and one-half to four weeks. Such results if due to ovary may be prolonged and may prove cyclical.

Parabiosis

Zacherl (477) has employed coli anastomosis in rats to study the effect of parabiosis. The estrual periods of the partners occurred independent of each other. If one rat became pregnant the cycles of the non-pregnant partner continued undisturbed. Conjunction with a male or with a castrated female produced cessation of cycles until the union was severed.

Kross (258) united pregnant rats near term and found that the onset of labor in one, did not affect the onset of parturition in the partner.

Corpus Luteum

Ablation Experiments

"Sensitization" of the Uterus for Nidation

The attention of the earlier investigators was focused for a long time almost exclusively on the corpus luteum. This was largely due to the fact that Prenant (379), Beard (37) and Born (Fraenkel, 127) regarded the corpus luteum alone as a gland of internal secretion and therefore sought explanation of all the sex phenomena mainly in the yellow body. Furthermore it was much easier and more feasible to shell out the corpus luteum than any other component of the ovary.

Prenant, Beard, and Born, all three, considered the effect of the corpus luteum inhibitory upon ovulation.

Ablation Experiments

Fraenkel (127)was the first to attempt to solve Born's hypothesis by experimental methods. He operated upon pregnant rabbits, removing the corpora lutea, finding with uniformity that if ablated early in pregnancy, the fetuses were absorbed, if later in pregnancy, aborted. Injury, excision or burning of other portions of the ovary did not produce this effect. Consequently Fraenkel felt justified in concluding that the corpus luteum was necessary for the nidation of the ova and the continuation of pregnancy.

> Fraenkel (127, 130), however, went further and stated that the corpus luteum produced menstruation. He has never fully receded from this stand but has, however, modified it. Recent work has definitely proved that the corpus luteum is not necessary to produce the menstrual phenomena. In both castrated monkeys and human females menstruation has been brought about by injection of female sex hormone obtained from follicle or placenta, the flow occurring when the injections were stopped.

Although much doubt has been cast upon Fraenkel's experiments which endeavored to show that the corpus luteum is necessary for the safe and continued nidation of the ovum, I feel that his claim in this connection is fullysubstantiated. Confusion has arisen because the lengthof the critical period, during which the yellow body is essential for nidation, varies so greatly in different species. In the rabbit this critical period exists during more than half of the duration of pregnancy and in the guinea pig during more than one-third of its duration. This does not prove that the same must apply to other species. Species variation in regard to time of sex phenomema are very striking, as was shown in the relation between ovulation and estrus. Therefore, the fact that in the human female a period of time of less than three weeks is critical for the presence of the corpus luteum, does not invalidate the theory at all.

> Innumerable observations fortifying Fraenkel's claim can be cited. The little known experiments of Wester (468) may be mentioned. In 5 pregnant cows the author squeezed out the corpora lutea through the rectum. In each instance, abortion promptly took place. In 5 controls, the more traumatizing interference of removing the ovary not containing the corpus luteum, per vaginam, by means of the ecraseur was performed and all 5 carried to term.
>
> Drummond-Robinson and Asdell, 1926 (101) found that every pregnant goat in which they removed the corpus luteum, promptly aborted.
>
> Hartman (207) in the opossum, noted absorption of embryos following removal of the corpus luteum.
>
> The literature referring to the human subject will be found in Ask-Upmark, 1926 (32). This author supplies a table with 51 cases of human pregnancies in which the corpus luteum was removed during the first two months without interruption of gestation. Four of these had the corpus luteum removed during the first month. They

are the cases of Essen Möller (Zbl. f. Gynäk. 1904, No. 28); Glissenoler, (Rev. Mens. de Gyn. and de Péd. 1911, No. 10); Lapthorne Smith, (Am. J. Obst. 1896, 34: 92); Roetter, Diss. Erlangen, 1918. Forty-one cases in the second month are collected by him.

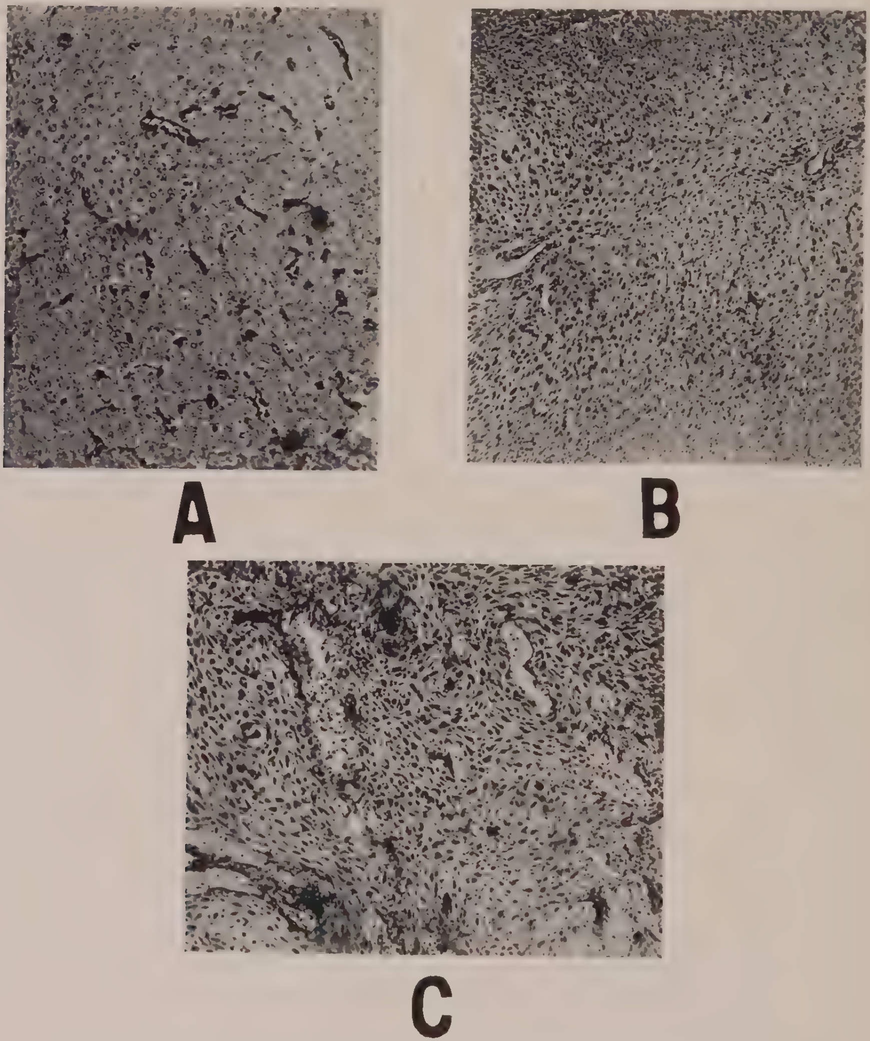


Fig. 21. Artificial Deciduomata. Ectopic Cast. (Photomicrograph.)
A. Deciduoma of rabbit. B. Artificial deciduoma of guinea pig. C. Uterine cast expelled during the course of an ectopic (tubal) pregnancy.

Deciduoma (Placentoma) Experiments

The main support of the Born-Fraenkel theory, showing that the nidation of the ovum is dependent upon the presence of the corpus luteum, was supplied by another type of experiment performed by Leo Loeb (298-300). Loeb determined the exact time of estrus in guinea pigs and rabbits by permitting sterile coitus after the Fallopian tubes had been tied. If, in a normal guinea pig or rabbit, from 2 to 9 days after coitus had taken place incisions were made into the endometrium, transitory tumor-like nodules developed which in microscopical characteristics were indistinguishable from the maternal portion of the placenta of the given species used in the experiment (Fig. 21). These tumors persisted for 10 days and then underwent necrosis. That this "deciduoma" or "placentoma" formation was due to ovarian action could be readily proved by the fact that after castration was performed no deciduomata developed. Going one step further, Loeb burned out merely the corpora lutea and then was unable to obtain deciduoma formation, though this formation was unaffected by similar or more extensive injury to any other portion of the ovary. Still more substantiation of hormone influence was adduced by the fact that auto-transplants of the uterus showed slight deciduoma formation in the transplanted portions, if the animal had functioning corpora lutea. A similar reaction could be obtained by other mechanical stimuli resembling that of the imbedding of the ovum. Foreign bodies introduced into the uterus of rabbits, for example, produced a similar stimulating effect on the endometrium and a similar placentoma reaction.

That this reaction of Loeb's is not confined to the rabbit and guinea pig was proved by Frank in 1911 (135) in post partum rats between 2 and 9 days after the birth of the young, placentomata being produced. The results in cats was negative.

Corner (75) in 1919 fully confirmed my results, likewise using rats post partum.

Long and Evans 1922 (320) succeeded in doing the same in rats in whom inhibition of estrus was brought about by means of mechanical stimulation of the cervix through the vagina.

Teel, 1926 (438) was able to produce placentomata in cyclical rats by arresting the cycle by means of anterior hypophyseal fluid. During this arrest deciduomata developed.

Brouha in 1928 (56) was able to do the same after arresting ovulation by means of anterior pituitary lobe extract. Deciduomata could be obtained if the uterus was traumatized from 4 to 7 days after ovulation.

Deciduomata in Castrates

For a number of years, since 1911, (135) I have in vain tried to produce artificial deciduomata in the castrate in whom an estrous reaction was induced by means of follicle fluid, corpus luteum, or placental extracts. This work of ours has been performed on rats, rabbits and guinea pigs.

As I have considered this group of experiments of the highest importance, I have spent much time on them, but always in vain.

A large number of guinea pigs were castrated in 1927 by Drs. Goldberger and Felshin and injected with female sex hormone. The

> uterus of these animals was incised at various stages of the artificially induced cycle, some being injected again after operation. Every variation of time and injection that could be devised was tried, but without success.
>
> In 1928 we repeated these experiments on a large series of rats and in addition attempted to add the effect of stimulating the cervix per vaginam, as described by Long and Evans (320). Again the results were negative.

Recently Weichert (467a) claims to have obtained positive results by first producing estrus by means of an active female sex hormone extract and then "sensitizing" the mucosa by means of Hisaw's corpus luteum extract. We have been unable to confirm these results up to the present time.

If Weichert's experiments are confirmed and substantiated this should be considered conclusive evidence, by another method of experimentation, that the corpus luteum elaborates a hormone which sensitizes the endometrium. This sensitization, as Leo Loeb (298) showed, produces the *maternal* part of the placenta (decidua).

The Decidual Reaction

A decidual change is regularly noted in those tissues of the mother which are in close relation with the products of conception. Actual contact is not essential, for in tubal pregnancy the uterine lining is often changed into an exquisite decidua although far removed from the ovum (Fig. 21C). Under these abnormal conditions the immediate tubal surroundings of the ovum show a decidual change in at most 15 per cent. (Fig. 22).

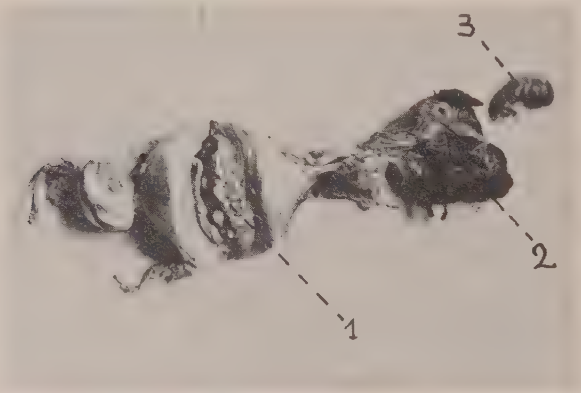


FIG. 22. HUMAN TUBAL PREGNANCY. RUPTURE OF TUBE.

1. Decidually changed uterine endometrium. 2. Rent in tubal wall. 3. Fetus extruded from tube.

How Long Is the Corpus Luteum Essential in Pregnancy?

From personal as well as collected data the following appears warranted:

In the human	14 days;	gestation 278 days	5 per cent
In the guinea pig	7 days;	gestation 61 days	11 per cent
In the rabbit	9 days;	gestation 30 days	30 per cent
In the bitch	30 days;	gestation 66 days	45 per cent

Inhibitory Action of the Corpus Luteum In the Absence of Pregnancy

Beard (37) believed that the corpus luteum suppresses ovulation. Loeb (298-301), agreed with this conception and offered the following experimental proof. The cyclical rate of a large number of guinea pigs was determined by means of the time at which they accepted coitus. The acceptance of coitus in these guinea pigs in whom the tubes were tied, determined the time of estrus and ovulation. He thereupon excised or burned out all the corpora lutea in guinea pigs whose length of cycle was normal, and found that the interval elapsing before the next estrus was definitely shortened. No such effects were produced by laparotomy, by laparotomy combined with excision or cauterization of other portions of the ovary. This work has been very generally accepted.

In human beings ablation of the corpus luteum within 4–5 days of the expected menstruation is regularly followed by an immediate menstrual flow. This is due to the fact that the prepared or "charged" endometrium disintegrates when the female sex hormone stimulus is abruptly withdrawn by removal of the corpus luteum, Vértes (454).

If the corpus luteum is removed earlier menstruation is delayed, because the mucosa is not yet fully charged and a new follicle must have time to ripen and burst.

Loeb's contentions have been strengthened in a more direct way by the experiments of Papanicolaou (356), Parkes and Bellerby (370), and Gley (185).

These recent experiments were based upon a different mode of procedure. The investigators determined the duration of the cycle in a large number of individual animals of a given species by means of the vaginal spread and then attempted to influence the cycle by corpus luteum extract injections.

> Papanicolaou (356) used guinea pigs injected with a lipoid extract of corpus luteum, the preparation of which was not described in detail.
>
> Parkes and Bellerby (370) employed mice and injected with an acetone extract.
>
> Gley (185) injected a "non-toxic" corpus luteum extract into cyclical rats.

These results all point to an inhibitory action on ovulation, no estrus taking place until the injections were stopped.

As the same animals may show great variations in the length of their cycles when untreated; as the cycle is readily disturbed by non-specific interventions such as handling, change of food and environment, operation, etc., these results must be accepted with some reserve. Another matter of doubt arises from the fact that some investigators employ the lipoid, others the water fraction with equal success.

> Hisaw (224, 225, 225a) using the virgin guinea pig in full estrus, injected a water soluble corpus luteum extract which, if potent, loosens the inter-pubic ligament within 12 hours. This test is based upon his discovery that in the pocket gopher during estrus and in pregnancy, the symphysis pubis is absorbed.
>
> Bencan, Champy and Keller (39) claim to have obtained a water soluble corpus luteum extract which produces merely congestion and not growth of Müller's ducts and the breasts.

We (Frank and Goldberger unpublished) have repeated some of these experiments. Papanicolaou's results could not be repeated as no description of his extract has ever been published. We have followed Parkes and Bellerby's technic minutely with several batches of material, in a large series, with uniformly negative results. We are still repeating these experiments.

The results with Hisaw's extract obtained by extraction of the corpus luteum, have occasionally produced loosening of the estrous guinea pig's pubic ligaments. Weichert's deciduomata in castrates, we were unable to confirm.

Persistence of the corpus luteum and consequent inhibition of ovulation is frequently noted in the non-pregnant cow (Tandler, 436). Veterinarians under these circumstances squeeze out the corpus luteum by rectal manipulation, estrus then promptly supervening (Williams, 471).

The fetus per se likewise, does not inhibit ovulation, as I showed (Frank, 135) by implanting minced rat fetuses, either untreated or previously treated with ether, (according to the technic of Askanazy (31) into the peritoneal cavity of rats. The "experimental teratomata" grew for varying periods of time in their host without inhibiting or affecting the cycle. If the fetus is absent, as in chorionepithelioma, ovulation, nevertheless is suppressed.

Lactation may or may not inhibit ovulation. In many species at least one ovulation occurs immediately post partum—rat, rabbit, etc. Even in man, impregnation within two days of partus has been recorded.

Parkes (359, 361, 363) has studied the effects of lactation in the mouse. Suckling appears to protract the life of the corpus luteum and to inhibit follicle ripening. By employing relays of nurslings this effect could be protracted far beyond the normal period of time. It requires 10 M.U. of œstrin to force estrus on the lactating mouse. If the ovaries (containing the corpora lutea) are removed only 2 M. U. are needed.

Most women do not menstruate during the early weeks of lactation.

After 8-12 weeks a considerable number begin to menstruate (Weinberg, 466). Some remain amenorrhoic throughout lactation. If lactation is unduly prolonged (1-2 years) permanent *lactation atrophy* of the uterus, accompanied by permanent amenorrhea, may supervene. (Thorn, 441).

We have not as yet studied the blood of amenorrhoic lactating women to determine whether a subthreshold blood cycle can be demonstrated.

For the present, therefore, this particular phase of the question in my opinion is still *sub judice*.

Inhibition by the Corpus Luteum, in the Presence of Pregnancy

Ovulation appears to be absent throughout pregnancy. The mechanism which brings about this arrest has been studied.

Other Possible Inhibitory Factors

Pregnancy per se is no absolute bar to ovulation. Leo Loeb (300) removed the corpora lutea of pregnant animals, which then ovulated although the pregnancy continued. Zondek and Aschheim, 1928 (488), by means of injection of large quantities of anterior lobe pituitary extracts, have succeeded in causing ovulation at all periods of pregnancy in the mouse, recovering numerous ova in the tube as Engle (104a) had done previously with implants.

My own views on whether the presence of the corpus luteum is necessary are as follows: The corpus luteum secretes at least two hormones. The first of these is the female sex hormone which is needed to produce enlargement and vascularization of the uterus in order to accommodate the growing ovum. The need for this function of the yellow body is extremely short in the human being, where we have (Frank and Goldberger, unpublished) found an unusually large amount of female sex hormone in the earliest chorionic vesicles which we could obtain. It appears that the chorion of other species contains less female sex hormone per gram than that of the human, throughout the term of gestation. This function of the corpus luteum is simply a continuation of the elaboration of the female sex hormone first produced by the follicle, and after the corpus luteum has become functionless, continued by the placenta. The other secretion of the corpus luteum apparently has two further functions. The first subserves the purpose of assuring sensitization of the endometrium with consequent decidual or placental reaction. As soon as firm embedding takes place, this function is no longer essential.

And lastly, an inhibitory action upon ovulation has been demonstrated. Whether this is a true specific and truly hormonal reaction remains to be proved. It is known, for example, through experiments of Zondek and Aschheim (488) that ovulation can be forced upon the pregnant organism by means of anterior lobe pituitary extract without the induction of abortion, and therefore this inhibitory mechanism, which may be well worth while from the point of economy (that is saving of unnecessary follicle wastage) appears to be otherwise unessential.

It is not at all surprising that the corpus luteum can elaborate not only female sex hormone but also an inhibitory fraction. The anterior pituitary also produces at least two distinct hormones.

Whether the granulosa lutein cells produce one fraction and the theca lutein cells another is a question that cannot be answered at present.

Since the completion of the manuscript two very important articles by Corner have been published. They complete the missing links in corpus luteum physiology.

Corner and Allen (74a), by means of an aqueous extract of the corpus luteum, were able to produce the special progestational uterine endometrial reaction in castrates such as Weichert had developed. In addition, Allen and Corner (12a) were able in rabbits to have fetuses develop and go to term, although the ovaries were ablated within 24 hours after copulation. Such development did not take place if corpus luteum extract was not injected.

Further proof that the corpus luteum secretes two hormones is offered by the following: Since completing the manuscript, my co-worker, Prof. Gustavson of Denver, has sent me two extracts obtained from the same batch of corpora lutea: the one an aqueous fraction prepared according to the directions of Hisaw which loosened the pubic ligament of the estrous guinea pig (and which is similar to the extract used by Corner); and a second fraction, lipoid soluble, which contained a large amount of female sex hormone.

I therefore feel justified in considering the aqueous fraction of the corpus luteum as uterus sensitizing and follicle inhibiting in nature, while the lipoid fraction contains the female sex hormone as previously determined by Frank and Gustavson.

CHAPTER IV

EXPERIMENTS WITH EXTRACTS OF THE FEMALE SEX HORMONE

AQUEOUS—LIPOID—FOLLICLE FLUID

FOR a number of years, in fact up to the year 1912, all of the extracts obtained from various portions of the sex glands, which were to be examined for their potency, were made with aqueous solutions, occasionally with addition of weak alkalies or acids, or of sodium chloride.

Aqueous Corpus Luteum Extracts were judged by the immediate phenomena produced after intravenous or subcutaneous injection. The work of Villemin (455) emphasized the fact that corpus luteum extracts were toxic and caused marked sinking of the blood pressure, convulsive seizures and death.

These toxic phenomena in 1910 I readily explained (Frank, 134) as due to intra-vascular clotting. By the preliminary injection of sufficient hirudin, which is leech extract, and which completely inhibits blood coagulation for a period of several hours, many times the lethal amount of aqueous corpus luteum extract could be injected into the veins without producing disquieting or harmful effects.

Adler (2) in 1912 obtained similar protection by means of hirudin against the dangerous by-effects caused by intravenous injection of ovarian and corpus luteum press juices.

Aschner (27) in 1913 employed watery extracts of ovary and corpus luteum in mature guinea pigs. The extract was not thermostabile. His results were inconclusive.

Gley, 1928 (185) still speaks of the toxicity of corpus luteum extracts.

Aqueous Placental Extracts

Veit (452) had based a very interesting theory explaining eclampsia upon the observation of Schmorl (403), who had actually demonstrated emboli composed of chorion epithelium and even complete villi in the maternal circulation, especially in the pulmonary as well as in the liver capillaries of the mother. In broad outline Veit's explanation was as follows. Schmorl has demonstrated syncytium in the lungs of the mother. Liepmann (282–3) has furnished the biological proof by the precipitin test that syncytial constituents are present in the blood of pregnant women, and this fact substantiates Veit's assumption that the placental proteins follow the laws of antitoxin formation and conform to the side chain theory. Kollmann (255) has shown by cytolysis *in vitro* that syncytium is dissolved by the maternal blood; this dissolution presupposes a solvent chemical substance in the blood—a lysin. Veit based all that was to follow upon the assumption "*that there is a continued entrance of syncytium from* *the intervillous space into the veins of the mother, and a subsequent lysis of the syncytium*" from the very beginning of pregnancy to its termination.

Liepmann (283) performed further experiments in substantiation of this theory. However, my own work (132–3) in which I not only was able to use the cytolytic and the precipitin reactions, but also the then newly discovered complement deviation test of Bordet and Gengou, did not substantiate these contentions. I found that the placenta produces only *species* and *not organ specific* reactions, thus practically cutting away the whole foundation of Veit's hypothesis.

Aqueous Fetal and Placental Extracts

Almost as important in stimulating the work on the endocrine glands as the erroneous experiments of Brown-Séquard is the work of Lane-Claypon and Starling (1906) (262). For one thing, these authors coined the term of hormone from "to carry a message." Lane-Claypon and Starling injected into normal rabbits aqueous extracts of rabbits' fetuses, of placentae, and of ovaries. According to them, the injection with placenta and ovarian extracts proved negative, while even Berkfeld aqueous extracts of fetuses produced a marked increase in the size of the breasts, an increase in size comparable to that of early pregnancy. These striking and inspiring results were confirmed by Foà (125) in 1908 who showed that cows' fetuses, when injected into rabbits, produced a similar growth activation of breast. Not long after that Biedl and Königstein (44) added further confirmation, still working with rabbits. Approximately simultaneous with our work, Aschner and Grigoriu (28) agreed with Starling's results.

Basch (35) claimed to have obtained milk secretion in multiparous rabbits by the injection of placental extracts and a similar effect in one non-pregnant bitch. His work, as I pointed out (136), failed to distinguish between milk secretion and activation of growth of the breast.

Stimulated by these most important observations, I started out with the collaboration of Unger, in 1911 (166), to continue the work of these authors in the hope of obtaining further insight into the physiology of the sex phenomena, as well as perhaps in elaborating a useful therapeutic agent, such as Basch had already put upon the market in his placental extract.*

> We therefore injected white rats with extracts from the fetus obtained from sows. Our work which was very carefully controlled, gave entirely negative results. We thereupon took rat fetus' extracts and injected them into a second series of rats with equally negative results. Although it seemed unlikely that a reaction of this kind should be limited to rabbits alone, especially after Foà's results, we found it expedient to repeat Lane-Claypon and Starling's experiments using their technic. We thereupon injected rabbits with Berkfeld

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* This and similar extracts have, however, long since been abandoned because they proved inert.

extract of rabbits' ovaries, rabbits' fetuses and rabbits' placentae. In our three rabbit series, the resulting breast changes were so discordant, the ovarian and hypophyseal injections apparently producing very active hyperplasia while the fetal and placental extracts caused no change, that we thought it necessary to study the normal physiology of the rabbit's breast.

Our results showed that a cyclical change in the virgin rabbit's breast occurs under the influence of the ovary. We proved this by taking immature, normal, virgin rabbits, excising a breast on one side, and preserving this breast by the simple gross technic outlined by Claypon and Starling (removing skin, staining in bulk with hematoxylin, clearing with oil, and preserving the specimen in balsam between microscopical slides (see Figs. 23–24). When such rabbits were kept under observation, after the onset of puberty, breasts being removed at different intervals, it was soon noticed that with the onset of puberty and with the occurrence of ovulation, if this took place, a tremendous growth of duct and acinar tissues occurred. The most marked acinar increase was noted at the height of estrus. In the interval, only the smallest ducts regressed, the acini likewise involuting, but the main duct system and their branches being permanently laid down. Thus we found an explanation not only for the changes described by Claypon and Starling, but also for the non-concordant results obtained in our series of rabbit experiments.

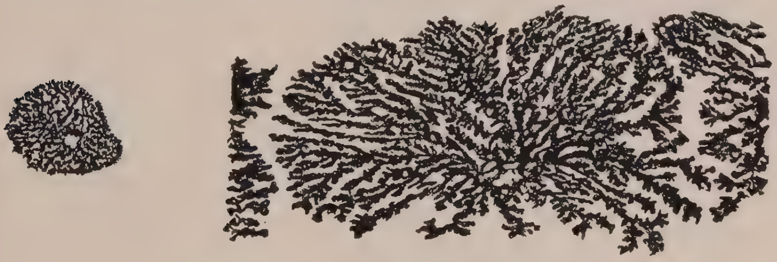

Fig. 23　　　　Fig. 24

Fig. 23 and 24. Normal Virgin Rabbit's Breasts.

Fig. 23. Breast of normal virgin rabbit before onset of first estrus.
Fig. 24. Corresponding breast of same rabbit after onset of estrus.
(Projected by means of Edinger apparatus on paper and drawn to scale.)

Our results also showed that even castration did not cause a rapid regression of the cyclical breast hyperplasia, that no proof had been offered to show that the fetus or placenta directly produced growth of the breasts in pregnancy, and that the evidence pointed to the fact that the persistent corpus luteum of pregnancy may produce this breast growth in pregnancy as had already been pointed out upon purely theoretical grounds by Tandler (436) and Foges (126).

We further stated that such evidence as existed made it unlikely that the fetus is at any time the controlling factor. In substantiation, we mentioned a fact known to clinicians, that the breast changes occur in hydatid mole and chorion epithelioma, without fetus, which fact Halban had already realized in 1905 (200).

Our main conclusions therefore were that the breast changes were due to the ovary and not to the fetus. I phrased this conclusion in a summary that same year (135) "at present it seems most probable that the maturing follicle causes the cyclical change, and that the persistent corpus luteum accounts for the hyperplasia found in pregnancy." Not until 1917 was I able to prove the influence of the follicle by experimental means. See page 73.

Our work (166) therefore not only contradicted Lane-Claypon and Starling (262), Foà (123), Biedl and Königstein (44), etc. but also explained how these investigators had arrived at erroneous conclusions by overlooking and not taking into account the normal cyclical breast changes. During the course of our investigations we came across the work of Bouin and Ancel, dating from 1909 to 1911 (13, 15, 16, 52, 53, 53a) in which these investigators produced ovulation in the rabbit by means of copulation with a vasectomized buck. By this clever method of experimentation in the rabbit, an animal which evinces no reliable external signs of estrus, they were able to determine the time of estrus. Their results agree with what we found, namely that the greatest hyperplasia of the breasts was coincident with the full flourishing of the corpus luteum. Bouin and Ancel therefore concluded that the breast hyperplasia is due to secretion of the yellow body.

The work of Lane-Claypon and Starling is still invariably quoted in connection with mammary growth, although all subsequent investigators have confirmed our (Frank and Unger) results and interpretations.

Others who worked with simple, nonconcentrated aqueous extracts (Ovarin, Poehl) were Adler, 1912 (2) and Aschner, 1913 (27). In consequence of the negligible amount of active substance injected, as well as due to the fact that adult guinea pigs and rabbits were used, their results were no more convincing and have never been confirmed. The work of Okinschitz (351) which falls into this group will be analyzed later (see p.73).

Halban's Placental Theory

Although not based upon experimental work but upon clinical observation, the main contentions of Halban have stood the test of time and experimental inquiry so convincingly that his work should be mentioned in this connection. Halban (200) considered the placenta a gland of internal secretion which, by its influence, caused the growth and hyperemia of the uterus and of the breasts throughout the duration of pregnancy. After the expulsion of the placenta during the third stage of labor, this influence is abruptly removed from the body. In consequence of this withdrawal, the mammary glands, which had been built up through the placental stimulation, are now able to secrete milk. In cases in which the placenta is retained with its circulatory communication with the uterine wall intact the activated breasts cannot secrete until the afterbirth is removed.

In addition, this placental stimulus is not limited in its effect to the mother, but likewise affects the fetus *in utero*. In consequence of this, the breasts of both male and female fetuses are stimulated in a fashion similar to those of the mother. The uterus of the female fetus is likewise influenced as is the prostate of the male. Therefore, after birth, in a certain number of cases, colostrum is noted in the breasts of both male and female infants, and, more rarely, the uterine endometrium of the neonatus shows a menstrual-like bleeding.

Halban likewise recognized that after the early period of gestation had passed, the ovaries could be removed without interrupting pregnancy or interfering with milk secretion post partum.

The fetus could likewise be dispensed with (death of fetus, hydatid mole) without affecting uterine growth or mammary secretion. The stimulus was not local but chemical and through the blood, as shown by the gravidity changes in the uterus in tubal gestation.

Almost every phase of Halban's work has been confirmed. Quite recently Otto Frankl (169) by experiment showed that no milk secretion took place in puerperal rats as long as fresh placental implants remained active in the host. This interesting confirmation was obtained by implanting fresh pieces of placenta in the subcutaneous tissue of white rats before or after they had borne their young.

Halban's observations have proved of important use to me in my clinical work, and I shall again refer to further confirmation of his theory in the second portion of this monograph (see page 248).

FERMENTS AND ENZYMES OF THE PLACENTA

Between 1911 and 1913 I was interested in the ferments and enzymes of the ovary and the placenta. It was by no means clear to me at the time whether at least some of the reactions noted in the sex cycle might not be due to enzymatic rather than hormonal influences.

In 1911, in the conclusion of an article on the "Function of the Ovary" (135) I reported on work performed together with the late Mr. T. Lefkowitz in which we compared the ferment content of the ovary and uterine mucosa of sheep and cows examined at different functional stages. For the sake of comparison and control, we selected the mucosa of the bladder, a comparatively inert organ, obtained from the same animal. Of the ferments studied the alkaline and acid proteases were found to be the same in the ovary and uterine mucosa of both pregnant and nonpregnant animals. No difference under these conditions was likewise noted in the erepsin contents. The lipase of the ovary remained the same in non-pregnant and pregnant animals, but the lipase content of the uterine mucosa was found greater in the non-pregnant. The amylase content of both ovary and uterine mucosa was greater in pregnant animals.

The last observation conforms with the opinion of Lockhead and Cramer (297) that the fetus obtains most of its carbohydrate nutriment from those areas to which the placenta is attached and which are in close proximity with glycogen stored in the decidua.

> It will be remembered that Halban and Frankl (202) injected tryptic ferments into rabbits' uteri and claimed by this means to have obtained changes resembling those of proestrus. They also found a tryptic ferment in the mucosa of the human uterus during the premenstrual phase and interval. My own attempts to duplicate their results convinced me that besides the erepsin found normally in the tissues, bacterial action accounted for the tryptic-like digestion observed by these authors.
>
> Rosenbloom, (395) in very early human chorion, with which I supplied him, and which was aseptically obtained, during the induction of a therapeutic abortion, was unable to demonstrate any enzymes whatever. We know from numerous observations since then, that the human chorion of this stage already contains a considerable amount of female sex hormone (see pp. 118 and 255).

It would thus appear that the ferment and enzyme content of, at least the placenta and probably of the ovary, does not play any important rôle in producing the sex phenomena.

This subject had been investigated by numerous workers. For the literature I refer to my article "An Experimental Study of the Placenta under Physiological and Pathological Conditions; The Ferments and Vital Staining," 1911 (137). Almost all investigators appear to have omitted the very necessary precaution of removing the large amount of blood circulating in the placenta. The impossibility of completely freeing the human placenta of blood had become apparent to me from previous work, 1906 (133). The original question at issue was whether the placenta was an organ of metabolism or merely an organ of absorption. My investigation showed that the main portions of the ferments found in the placenta were accounted for by the contained maternal and fetal blood. Furthermore, changes in function were unaccompanied by sufficient changes in ferment content to be demonstrated by any known methods. It was noted that even small fetuses contained an appreciable amount of ferment and that the organs of larger fetuses could show larger ferment values than those of the corresponding organs of the mother. The evidence obtained by "vital" staining likewise favored the view that the placenta is a passive organ for exchange rather than an active organ for metabolism. No evidence was obtained by this method that any of the pregnancy changes were due to the ferments of the placenta.

Lipoid Extracts of Ovary and Placenta

An entirely new impetus to the study of the female sex hormone was given by Iscovesco (230) who from 1908 to 1914 published numerous papers dealing with the effect of lipoid extracts obtained from various organs. Our attention will have to be confined to the extracts obtained from the ovary and placenta.

Iscovesco's publications first (231–2) dealt with a lipoid extract obtained from the whole ovary and corpus luteum, his second (232–241) with lipoid extracts of the placenta. These extracts, when injected into mature, isolated (and therefore not cyclical) rabbits, produced marked hyperplasia and congestion of the uterus and vulva, as well as of the breasts. These growth phenomena were so striking as to be readily recognized.

For the technical details published by Iscovesco see page 129. Here it will suffice to say that the fresh minced tissue was extracted in alcohol and that further extractions were performed with other lipoid solvents according to a definite technic some part of which has formed the basis of practically all of the work performed since then.

Effect on Uterus and Breasts of Adults

The concentrations obtained by these means were so striking as to convince investigators that the previous work performed with press juices and aqueous extracts was negligible. In direct succession after Iscovesco's first publication, Schickele, 1912 (401); Fellner, 1912–3 (113–4); Aschner, 1913 (27); Herrmann, 1913–5 (217–8); Seitz, Wintz, and Fingerhut, 1913 (414); Frank and Rosenbloom, 1915 (165) experimented with extracts obtained with lipoid solvents. One and all agreed that whether rabbits, guinea pigs, or dogs were employed, striking and unmistakable stimulation of the tubular and mammary systems of adult females could be obtained with lipoid extracts of the whole ovary, the corpus luteum, or the placenta.

Of these investigations, Fellner's (113–4) 1912–3, where the most complete.

His extracts prepared by extraction with alcohol, petroleum ether and again alcohol were emulsified with water.

Injected into virgin and castrated rabbits and guinea pigs, he obtained marked uterine and breast reactions. His conclusions hold good today.

1. In the placenta, membranes and corpus luteum containing ovaries are materials (Stoffe) which by subcutaneous or intraperitoneal injection produce growth of the breasts, nipples, and uterus; also estrus and pregnancy phenomena in the uterine mucosa, pregnancy changes in the vagina.
2. Milk secretion was not noted.
3. The substance goes into saline solution, is alcohol, ether and acetone soluble, may be a lipoid.
4. Excised guinea pig uterus responds to aqueous alcohol-ether extracts of placenta with a strong protracted contraction.

The details of the experiments of Frank and Rosenbloom (165), never published *in extenso*, were as follows:

35 castrated rabbits and 8 castrated white rats were used. Of the rabbits, 5 were very young and immature (500 g. weight or less).

12 rabbits injected (from 11 to 22 times with 0.1–0.2 g. amounts) with placental extracts;

10 with placenta lipoid soluble = all positive

2 with placenta lipoid insoluble = all negative

18 rabbits injected (from 10–27 times with 0.2 to 0.3 g. daily) with corpus luteum extracts.

13 with corpus luteum lipoid soluble = 5 positive, 5 weak reaction 3 negative*

5 rabbit controls = all negative

8 castrated rats were used.

3 ats were injected with 14 injections (0.01–0.02 g. daily) of placentra soluble = 2 positive, 1 weak.

3 rats were injected with corpus luteum = 3 negative*

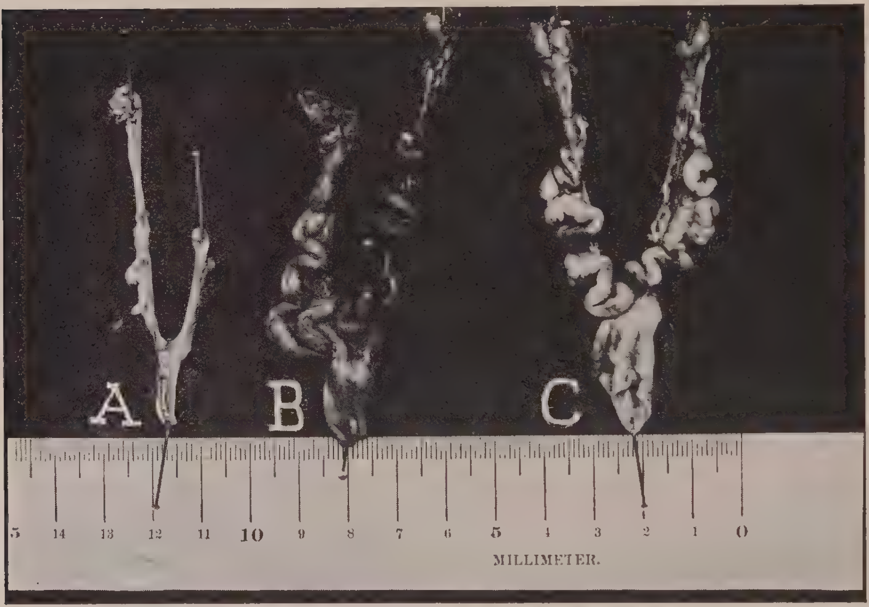

Fig. 25. Experimentally Induced Growth of Rabbit's Uterus.

A. Control castrate of same weight as B and C. B. Uterus of castrate stimulated with placental extract. C. Uterus of castrate stimulated with corpus luteum extract. These animals received daily injections over a period of two weeks.

2 rats control = all negative.

A *positive reaction* signified marked increase in size of the uterus and vagina, corresponding to at least twice the diameter of the horn removed for control at the time of castration.

A *weak reaction* signified full arrest of castration atrophy of the uterus.

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* The corpus luteum was obtained in four batches. All the negatives occurred in two batches "degreased" by mistake at the packing house.

Effect on Induction of Premature Puberty

Aschner (27), Fellner (113–114) and Frank and Rosenbloom (165) called attention to the fact that the sexual system of immature animals could be rapidly stimulated by these same means, thus producing premature sexual maturity.

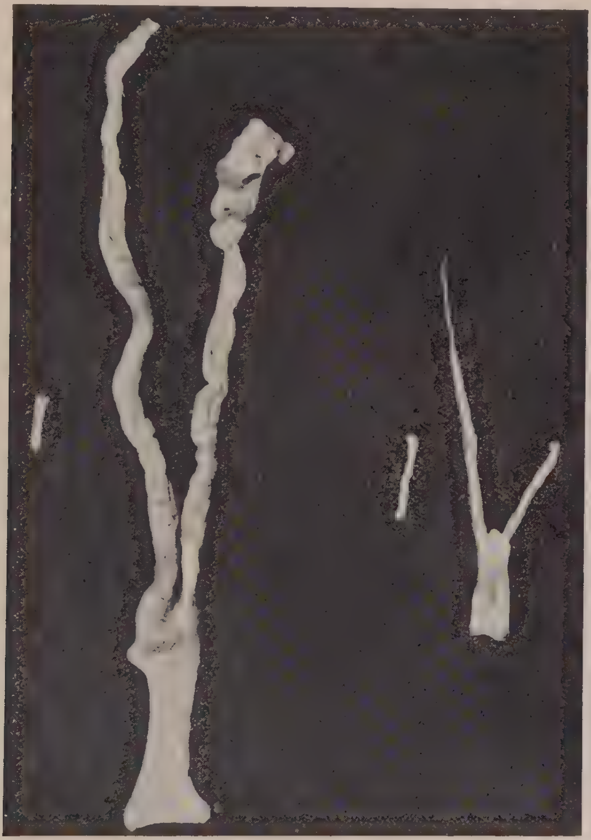


FIG. 26. RABBITS' UTERI, INJECTED WITH PLACENTAL EXTRACTS.

To the left, uterus of castrated rabbit, stimulated with acetone soluble placental extract. The small fragment to the left represents the piece of uterus removed as control at time of castration.

To the right is the uterus of a rabbit of equal weight. The animal injected with the acetone insoluble fraction of placenta. It will be noted that the portion of uterus removed for control is the same size as that portion removed at autopsy because the acetone insoluble fraction is inactive.

Effect on the Ovaries

With the exception of Herrmann and Stein (219) all investigators recorded that the ovaries were not affected by placental or corpus luteum extracts. Herrmann and Stein however, reported that their extracts increased the maturation of follicles in the ovaries of both immature and mature rabbits, followed by no ovulation but rather by a polycystic condition. Recently I note that Loeb and Kountz (304) 1928, report the acceleration of maturation of follicles resulting from the injection of follicle extract into immature guinea pigs.

Early Chemistry, 1915–1917

At first investigators were satisfied with crude concentrates. Soon, however, attempts at further purification were made. Of these the best known and most often quoted are those of Herrmann (217–218) who, by a combination of selective lipoid solubility, purified placental extracts up to a certain degree, and continued the purification by means of vacuum distillation. It was noted that the most active substance distilled over at the high temperature of 193 degrees centigrade. Herrmann considered his end product a lipo-protein (see p. 130).

Fränkel and Herrmann (168) next published a method of purification of corpus luteum and placental extracts, which they used as the basis for patents obtained in the United States, England, and on the continent of Europe. By means of further lipoid extraction and distillation they obtained a purer product from which all sulphur, nitrogen and phosphorus had been removed. The sole remaining components of the active substance were carbon, hydrogen and oxygen. Their ultimate analysis showed carbon 81.33 to 81.62 percent and hydrogen 11.32 to 11.49 percent. Their product, which they considered chemically pure and which they called a cholesterol derivative, gave a cholesterol reaction.

That the claims of having obtained the active substance in a pure state, made by Herrmann and Fränkel (168) and by Herrmann (217–8) alone, were unjustified and fallacious, was definitely proved by me in 1917 (141). As early as 1916, in work performed with Mr. Lee, we found that the activity of the specific substance continued unaltered after every trace of cholesterol and cholesterol reaction had been removed by the digitonin method. This therefore definitely showed that Fränkel and Herrmann's patented product was a mixture, and not a pure substance. Our observations have been confirmed repeatedly since then. (Doisy, Ralls, Allen and Johnston, 93).

By 1917 my chemical researches, performed in conjunction with Mr. Lee and Mr. P. M. Giesy had shown that the active substance, whether obtained from corpus luteum or from placenta was a dark, semi-liquid mixture. It resisted temperatures of 350° Centigrade. It likewise resisted the application of strong acids and strong alkalies for long periods of time (20 per cent H_2SO_4 and 20 per cent NaOH up to 12 hours in the presence of heat). No change in physiological activity was noted after prolonged saponification. On the other hand, activity was rapidly lost if the substance was exposed to oxidizing agents. We were then as yet unable to determine whether this was due to oxydization or polymerization. The active extract was fully soluble in 95 per cent alcohol, but less and less soluble as the strength of the alcohol was reduced. A partial solubility was noted in chloroform, ether, oils, and benzin.

The minuter details of these chemical researches are discussed on p. 131.

This brings me to the period in which I first experimented with follicle fluid. Before touching upon this subject I shall attempt to summarize the work performed by me antedating 1917.

Summary of Experiments Performed by the Author with Lipoid Extracts of the Placenta and Corpus Luteum

In May 1917 I was obliged to stop all experimental work for a long period. Five years elapsed before I could resume my work. For this reason I shall summarize the chief results obtained by me during or before 1917 in order to save the reader from having to search for them in the scattered literature.

1. No difference in physiological effect was noted between extracts derived from the corpus luteum or placenta, except that to obtain the same end results larger doses of corpus luteum than of placental extracts had to be employed.

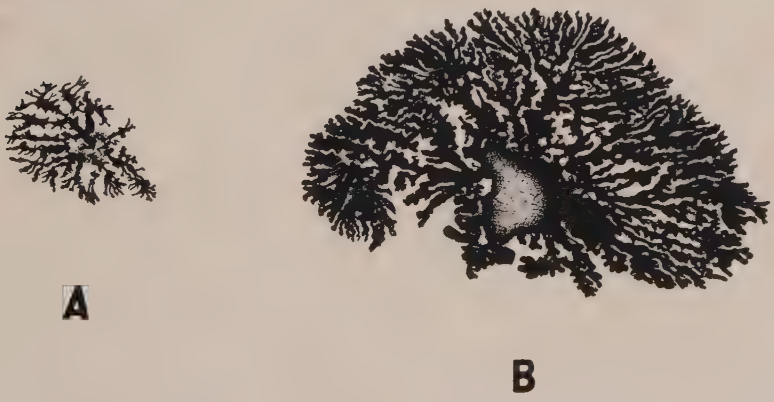


Fig. 27. Increase in Size of Castrated Rabbit's Breasts Due to Injection of Lipoid Placental Extract

A. Control breast removed at time of castration.
B. Corresponding breast removed at autopsy after series of injections.

2. Injected into immature animals, whether castrated or not castrated, rapid enlargement of the uterus, vagina, and breasts were obtained in rabbits and guinea pigs without any accompanying change in the ovaries. This induction of premature sexual maturity occurred in from 6 to 12 days, depending upon the size of the dose and the frequency of the injection.

3. Identical effects were obtained in mature castrates, the hyperemia and hyperplasia of the endometrium and uterine musculature reaching the proportions seen in estrus after 8 to 10 days of injection, and attaining

early pregnancy or pseudo-pregnancy dimensions if the injections were continued 4 to 6 days more. The gland changes in the endometrium, as well as the stromal changes, could not be distinguished from those noted during estrus and early pregnancy.

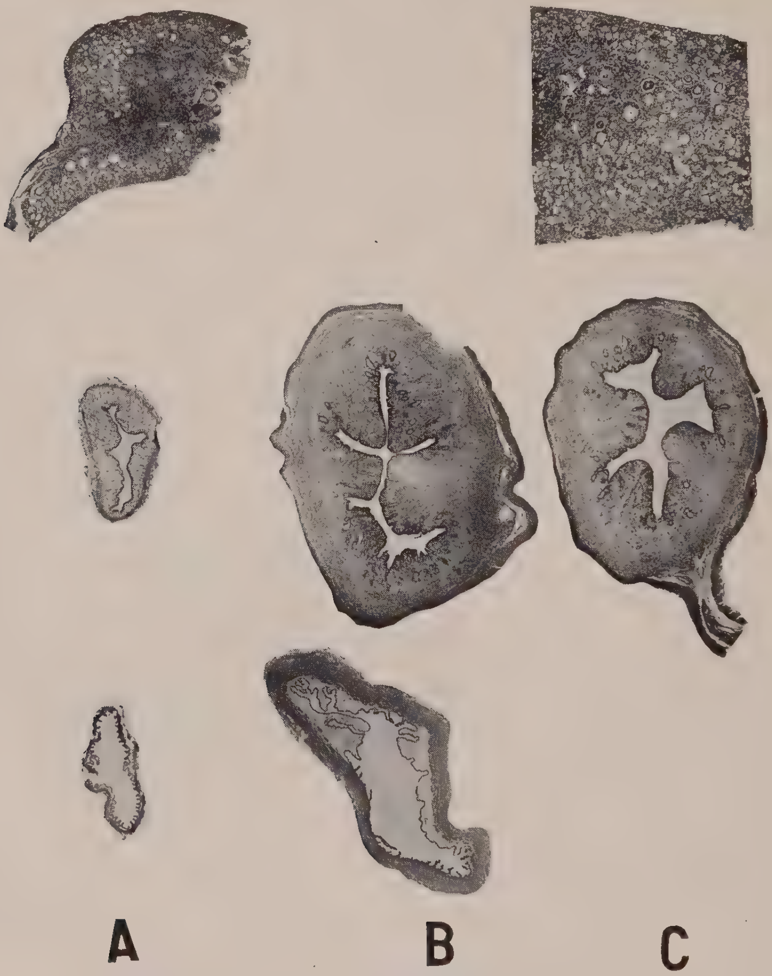


Fig. 28. Effect of Stimulation of Rabbit's Genital System.

A. Immature virgin rabbit used as control. Above, ovary; in middle, cross section of uterus; below, cross section of vagina.

B. Castrate injected over one week with female sex hormone. Above, cross section of uterus. Below, cross section of vagina. Note tremendous increase in size over that of control (A) of similar weight.

C. Immature virgin rabbit treated with same extract as B. Note above that injections have had no effect on the ovary and that the uterus has undergone the same stimulation as that of the injected castrate.

4. The breast changes corresponded to the normal cyclical changes occurring in mature virgin animals, and could be exaggerated in rabbits to a degree indistinguishable from the breast dimensions noted on the 10th day of pregnancy.

5. All of these changes were induced and studied in castrates, but because of the tremendous volume of operative work required in castration, I later used immature virgin rabbits after having convinced myself that the presence of immature ovaries did not in any way influence the reactions or the results obtained. Rabbits were always found immature if below 800 grams in weight.

6. It was noted that even prolonged injections of either corpus luteum or placental extracts, which so thoroughly and rapidly activated the tubular system and breasts, were entirely without effect on the ovaries of either immature or adult animals. The effect on the adult ovaries could best be studied in the isolated rabbit in which spontaneous ovulation does not occur.

> Heape as early as 1905 had discovered that ovulation in the rabbit normally occurred only after coitus. Hammond (203–4) has confirmed this observation. Bouin and Ancel (53) found that if a female was caged adjacent to a vigorous male, ovulation might take place (apparently due to psychical excitation).

7. As examination of the uterus involved either laparotomy, or more commonly sacrifice of the animal, I found it of use also to watch the growth changes occurring in the breasts and nipples of immature castrates and therefore mentioned this as an optional (Fig. 29) useful test for assaying the potency of extracts (141).

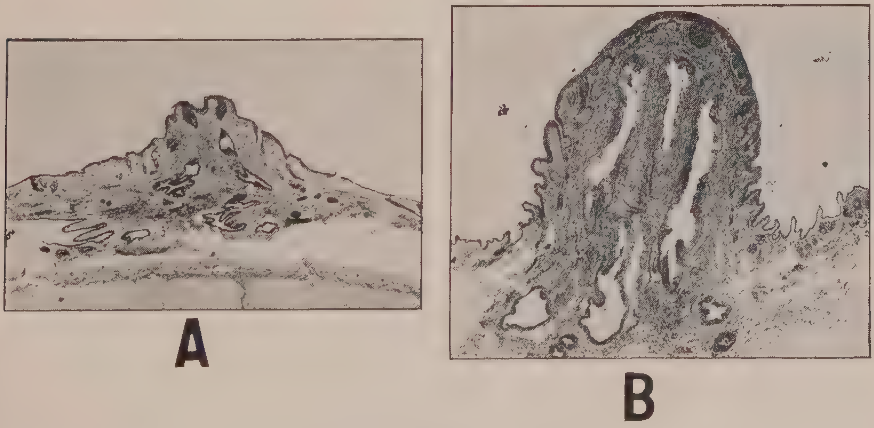


Fig. 29. Breasts of Rabbit.

A and B represent cross-sections through the nipple of correspondng breasts of same rabbit before and after treatment with female sex hormone. A. Breast removed for control. B. Huge increase due to daily injection of female sex hormone over a period of one week.

Steinach *et alia* (1928) (430) using albino guinea pigs, have recently published this method with some excellent colored illustrations, as a new contribution, and a new test for determining the potency of extracts.

8. In 1916 and 1917 (139, 141) I noted that transplanted fragments of uteri underwent hyperplasia at their new site (usually subcutaneous, in the breast or dorsal region) if potent extracts were injected from the time of transplantation on. Moreover the number of "takes" in a castrate were greatly increased if the transplants were stimulated by means of a potent extract. It was found that although the growth impulse given to these transplants could be kept up indefinitely by continuing the injections, the response always stopped before abnormal growth phenomena could be elicited. In other words, these stimulated tissues always retained normal morphological groupings. The utmost stimulation resulted in small transient, pseudo-tumors, resembling adenomyomata.

Jacobson, 1926 (243) at the instigation of Sampson, injected fragments of endometrium intraperitoneally into rabbits. He found 84 per cent of takes if during estrus, approximately 33 per cent during pregnancy, but only about 15 per cent in anestrus.

9. I found that castration atrophy of the utmost degree could be counteracted and full stimulation of the uterus obtained in castrates even after long periods of time had elapsed.

In one rabbit, for example, castrated 16 months before (141), a period of time which would correspond to several decades in the life cycle of a human being, a uterine condition resembling the early stages of pregnancy was induced by means of injected lipoid extracts.

The fact that a completely atrophic uterus retains sensibility and response for long periods of time, to the female sex hormone, is of importance in many ways, especially clinically. It encourages the hope that infantilistic genitals can respond to therapy in later adult life, and illustrates why long continued periods of amenorrhea may be succeeded by resumption of menstruation (see page. 220).

10. It was noted that a distinct increase in refractoriness to stimulation occurred as the maximum activating dosage was approached (139). This signified that small dosage would produce definite uterine and breast stimulation in the castrate and immature, that proportionally greater dosages were required to produce the effects corresponding to estrus (or as I at that time less accurately termed it "rut"), and that a proportionately still greater dosage was needed to produce the early pregnancy changes. An increase of dosage beyond these limits was entirely ineffective.

Laqueur in 1927 (271) wrote an elaborate article in which he fully confirms these observations of mine and amplifies them in many ways.

> On the other hand, I must disagree with those who attempt to differentiate between a "growth producing" and an "estrus producing" factor in the female sex hormone. These differences, in my opinion are the result of differences in dosage.

11. For many years Eppinger, Falta, and Rudinger (106), had theorized upon the effect of the interaction of various glands of internal secretion upon each other. The question arose as to whether the presence of other glands was essential to response of the sex system. Therefore I (141) ablated various glands of internal secretion, following this by injection of potent extracts, in order to determine whether the uterine and breast effects produced were in any way directly dependent upon the other glands of internal secretion. Pancreatectomy, adrenalectomy, thyroidectomy, as well as adrenalectomy and thyroidectomy in the same animal, were performed on rabbits. Some of the castrates survived sufficiently long to allow me to test the effect of potent extracts. In all of these no diminution of effects upon the uterus and breasts were noted.

The completeness of gland removal was verified in each case by autopsy and serial sections of the original site of the ablated gland or glands. These results signify that as far as the female sex hormone is concerned, the local effects appear to be independent of the presence or absence of other glands. These results, however, do not in any way mitigate against the possibility of interrelation under normal conditions. In fact such interrelation has been proved in regard to the ovary and anterior hypophysis (see page 14).

In 1928 Rathery, Kourilsky and Laurent (383) confirmed my statement that the female sex hormone works in the absence of the pancreas. They used folliculin. In the female depancreatized dog, folliculin moreover increases and prolongs the hyperglycemia.

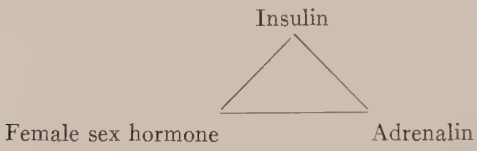
Zondek and Aschheim (487) removed the adrenals in mice, the cycle continuing, thus also verifying my results in reference to this important endocrine gland.

> For technical reasons I found it impossible to perform hypophysectomy with success, and therefore the question of whether the deprivation of this important gland affected potent extracts in a different fashion than those precedingly mentioned, remained open until 1927 when Philip Smith decided the question by successful hypophysectomy, and noted regular response to the female sex hormone in spite of this lack.

Prof. Heinrich Poll of the University of Hamburg delivered a lecture at Mt. Sinai Hospital, New York, on January 17th, 1929, in which he summarized the work performed by his group (including Blotevogel, Faure, Dohrn, etc.). He drew attention to the interrelationship between adrenal and ovary, ovary and pancreas, pancreas and adrenal as gauged by the effect on the chromaffin cells.

In summary his conclusions were:

> 1. That *Insulin* injections empty the adrenal cortex of chromophyl cells quantitatively pro unit injected.
>
> 2. Castration empties the chromophyl cells of Frankenhäuser's ganglion (cervical ganglion) of the test mouse. Female sex hormone, whether derived from animal or vegetable sources, restores the cells quantitatively.
>
> Pregnancy up to term increases the percentage of these cells in the cervical ganglion.
>
> 3. Female sex hormone injections empty the islet cells of the pancreas of their mitochondria.
>
> To illustrate the interrelationship he revived a modified version of the famous endocrine triangle.



This interesting hypothesis deserves consideration and study. For details of his test, see page 97.

12. Pharmacological investigation had shown that the non-pregnant virgin cat's uterus relaxes when adrenalin solution is added in the moist chamber; the pregnant cat uterus, under these conditions, contracts. In conjunction with Prof. Lieb, in 1916, in the Pharmacological Department of the College of Physicians and Surgeons, a few experiments were performed in which the uteri of virgin cats were stimulated and caused to grow by means of injection with an active lipoid extract of placenta. The uteri were removed, suspended in a moist chamber and adrenalin added. The resulting reaction was unchanged, namely that of the virgin cat's uterus. The exact interpretations of these experiments are not clear.

> Recently van Dyke and Gustavson (451) have again performed similar and more extensive experiments with somewhat different results. All the uteri, even after full activation and hyperplasia due to potent extracts, relaxed upon stimulation of the hypogastric nerve, with the exception of one which had been treated with corpus luteum extract "rich in estrous-producing hormone." This one uterus contracted as does the normal pregnant uterus.

The Effect of Follicle Fluid

According to Wester (468), Zanger in the year 1859 (Schweizer Arch. f. Tierheilkunde) first suggested ovulation as the cause of estrus and pointed to the persistence of estrus as the result of cystic ovaries in the cow.

Bucura, in 1907, (63) reported a single experiment which to me has always appeared of fundamental importance. He never pursued the subject further as far as I could determine.

> In the course of ablation experiments, a minute fragment of ovary was left behind in a rabbit in whom full estrous symptoms developed. At autopsy, confirmed by microscopical examination, it was found that the minute fragment consisted solely of a single maturing follicle without either corpus luteum or interstitial tissue.

This observation of Bucura's, in addition to the fact that full estrus develops in most animals before the rupture of the follicle, and the formation of the corpus luteum, induced me in 1911 (135) to state "before our knowledge of the female sex organs can be considered at all satisfactory, we must know whether maturation of the Graafian follicle causes proestrus."

The "acute" experiments of Sonnenberg (427) performed in 1907 were unknown to me. His mode of experimentation, in which effects were looked for within 30 minutes after the injection of follicle fluid, precluded the observation of any positive results.

The work of Okinschitz, 1914 (351) who injected aqueous and weak alcoholic extracts (approximately 25 per cent alcohol) of "the product of the secretion of the follicular apparatus" of animals (calves and immature pigs) appeared negative to me after careful reading of his protocols and scrutiny of the illustrations.

In spite of the few negative reports noted, in 1917 I determined to put this crucial question to further experimental proof. The first set of experiments was completed in May 1917, but for personal reasons, which will be referred to later, the publication of results was deferred until January 1922. (143).

At that time (1922) I stated the following:

> "In May, 1917, assisted by Giesy, I performed the following as yet unpublished experiments. Since then opportunity to continue the work has been lacking.
>
> A quantity of follicle fluid, obtained by puncturing ripening follicles from the ovaries of cows, was collected. In the one series, the ovaries were derived from non-pregnant animals, in the second from those of pregnant ones. Two virgin rabbits were injected subcutaneously in each series with 1 to 2 c.c. respectively, of the follicle fluid daily for ten doses. In all four animals, well marked hyperplasia of the uterus was noted, the material from non-pregnant animals being the more effective."

This experimental confirmation enabled me to state in the same article categorically that the so-called menstrual or monthly cycle is due to follicular action on the uterus.

> "as far as can be determined at present, only one factor comes into play, and that is the development, growth and evolution of the ovarian follicle, which begins as the primordial follicle, is called a Graafian follicle as it ripens, and, after ovulation or bursting has taken place, completes its cycle of existence as the corpus luteum."

> Throughout these stages the follicle produces a hormone which directly influences the uterus. The amount of uterine reaction appears to depend on the quantitative output of the secretion. Only during the early active stage of existence of the corpus luteum is an additional influence exerted on the uterus. This special function which facilitates nidation, i.e., the embedding of the ovum, will be referred to later."

It will be noted in this connection that then, as well as since that time, I have drawn no sharp distinction or demarcating line between the follicle and its successor, the corpus luteum.* The logical conclusion to be drawn from this was stated in another paragraph.

> "Pharmacologic researches, therefore, show that the follicle as well as the corpus luteum secretion can maintain the growth and nutrition of the uterus. In large dosage, hypertrophy of uterine muscle and mucosa takes place. The external genitals and mammary glands are also stimulated. These reactions are specific."

A year later, on September 4, 1923, (Frank 145) before the Colorado State Medical Society, I stated that the follicle fluid exerts the same effect as the lipoid concentrate of the ovary (the corpus luteum extract). This effect was at that time described as follows:

> "The lipoid concentrate of the ovary given to castrated animals whose genital tract has undergone definite atrophy, within a week's time produces huge hyperplasia of the genitalia, comparable to exaggeration of the reaction noted in "rut." Given to immature animals, the extract causes sexual development, including growth of the breasts. *In 1917 I found that the follicle fluid exerts an identical effect.*" (This sentence has now been placed in italics.)

I then analyzed the reasons that active ovarian or placental extracts have not been placed on the market. Among these reasons I gave

> "the follicle fluid, as can readily be understood, is time-consuming to collect and therefore costly. Unless the profession will insist upon a potent drug, they will never receive it."

Thus the reader will note that before September 8, 1923 I had already put myself clearly on record, and had demonstrated that the follicle fluid contained an active substance which produced in immature animals changes characteristic of rut phenomena.

On September 8, 1923, an article of utmost importance, entitled "An Ovarian Hormone" was published by Allen and Doisy (6). These authors reported that the follicle fluid from the pig's ovary, when injected into castrated mature and immature mice or rats, produced full estrus, hyperemia, growth and hypersecretion in the genital tract. They obtained these same results with Berkfeld filtrate and as well as with lipoid extracts of the fluid. The authors emphasize the fact that premature sexual maturity, as well as awakening of the sex instinct and copulation, could be induced by these extracts.

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* In a recent paper Ancel and Bouin, 1926 (16), likewise emphasize this continuity of action of the follicle and its successor, the corpus luteum. In a rabbit at estrus, follicle action produces the uterine changes. After "sterile" coitus (with vasectomized buck) is permitted, the corpus luteum increases and protracts these uterine changes (pseudo-pregnancy).

Their publication fully confirmed my contention and amplified my findings. *By far the most important item in their report, however, was the publication of a new method of assaying and titering the female sex hormone, depending upon the examination of the vaginal spread of living, castrated rats and mice.* In the castrated, untreated rat, the vaginal spread is composed almost entirely of leucocytes. The vaginal spread of an animal brought into artificial estrus, on the other hand, is readily distinguished from the preceding by being composed of squamous, non-nucleated scales. This new test has proved of greatest importance in the study of sex phenomena and sex physiology. Due to its simplicity, ease of application and cheapness, it has attracted many investigators into this previously uncrowded field. The full significance and importance of the vaginal smear test will be taken up in the next chapter. (See page 88).

Questions of priority are rarely of general interest. If merely the point of priority were at stake, I might feel inclined to let this matter rest, but as unfortunately, Doisy and Allen (93) have seen fit "*to cast doubt*" upon the work of all of those, including my own, who have obtained positive results with corpus luteum by whatever method, and have so regularly brought up the question of my having failed to recognize the importance of my discovery in their subsequent articles, I feel both justified and obliged to discuss this subject in some detail.

Before actually dealing with the subject in question, I want to repeat once more that I consider the publications of Allen and Doisy of fundamental importance and among the most valuable contributions to sex physiology. Their results, in my opinion, with the exception of their inability to demonstrate the active substance in the corpus luteum of any species except the human, have moreover been accurate, and most convincingly presented. Throughout their investigations, however, which were conducted with greater ease and speed than those of their predecessors because of the valuable Allen and Doisy test, the work of all others has consistently been referred to with scant acknowledgment and scant credit.

> In their first publication on the ovarian hormone (1923) (6) I was misquoted in that the authors spoke of my having induced uterine "hyperemia" instead of "hyperplasia" as I actually mentioned. They further stated, and they were entitled to hold this personal opinion, if they so chose, that "*one cannot be quite sure that the changes described were not caused by the activity of the ovaries. Previously, Frank and several writers mentioned by him (especially Herrmann) had reported similar results from the injection of material obtained from the corpus*

> *luteum and the placenta (Herrmann spayed some of his test animals). Consequently, this test does not seem to be specific for the hormone of the estrus."*
>
> The italics are mine.

I desire to point out the fact that I consider that Allen and Doisy are in error when they state that their test is specific for the hormone of estrus, because as I shall mention later, this same reaction is produced during pregnancy by the same agent, the female sex hormone, and has been elicited with the blood, urine and bile of males.

Moreover, *there is no "hormone of estrus."* Estrus is simply the culmination of the pregravidity changes, and is the time at which, in the lower species, coitus is permitted. There is no more a hormone of estrus than there is a hormone of proestrus or of pregnancy. One stage of the fertile sex cycle merges imperceptibly into the next, beginning with the end of anestrus and ending at the time of labor.

Other doubts in regard to some of the statements made in this article of Allen and Doisy were voiced by me in a letter to the editor which appeared in the Journal of the American Medical Association on September 29, 1923 (144). In this I called attention to the fact that the effects of follicle fluid corresponded in every way with the effects of the extracts previously used by other investigators, although these were obtained from the corpus luteum and the placenta. I should have been glad to let the matter rest then.

I have selected only a few examples out of many, to illustrate the regard accorded by Allen and Doisy to the work of others.

> In 1924 in the article entitled "The Extraction and some Properties of an Ovarian Hormone" (93), after mentioning Iscovesco, Fellner, Okinschitz, Herrmann, Fränkel, and Herrmann, and Frank, they sum up the situation by stating "although all of the authors referred to in the preceding paragraphs used lipoid solvents, many have obtained active extracts from whole ovaries or placenta, their finding of the hyperplastic substance in the corpora lutea casts doubt upon their observations."

To me it seemed rather extraordinary that two newcomers in this field of endocrinology, no matter how important and praiseworthy their own contributions proved to be, should be ready to *cast doubt upon the observations of all of their predecessors* because they (Allen and Doisy) could not obtain positive results with corpus luteum by means of their test.

It may be of interest to note that several years later Allen, Pratt, and Doisy (11) were able to find the active substance in the human corpus luteum and thereupon revamped their theory so as to conclude that the human corpus luteum must be regarded in an entirely different category from that of every other mammal!

In another paper, Allen and Doisy (9) in the discussion of my results, say: "It will be noted that the test animals were 'virgin rabbits,' *presum-*

ably adults (the italics are mine) with ovaries intact; consequently no proof is offered here that the liquor was a substitute for ovarian function."

This remark about "*presumably adults*" is really pure *presumption*, because I had taken pains in a letter to the editor of the Journal of the American Medical Association, previously referred to (144), to point out that I had used *immature* animals, long before estrus could occur under normal conditions, and in that fashion had avoided the confusing factor of ovarian activity. That Allen and Doisy could have overlooked this letter is quite out of the question as on page 1808 of this same journal (7) they, in reply to my letter, offer apologies for another misstatement, namely for having used the word "hyperemia" instead of "hyperplasia," thus entirely altering my meaning. They continue (9) that "in an earlier paper Frank concluded from one positive experiment, after other results had been negative, that he had demonstrated an active substance in extracts of corpora lutea." *The one positive experiment referred to*, however, consisted of a considerable *series* of animals, not of one animal, as is implied. (For Protocols see page 63.) Immediately after, they state in footnote 4, "in this paper the *admission* (the italics are again mine) *is made in a footnote that some of the corpora lutea were degreased before his preparations were begun*." Their phraseology is to say the least, unfortunate, for the late Dr. Rosenbloom and I were careful *ourselves* to draw attention to the fact (by the way, not in a footnote, but in the text proper) that by error, Armour and Co. had degreased two batches because it had been thought (by the packers) that this would make the substance more stable. We also, ourselves, had then in 1915 drawn the inference that "naturally, in the process of degreasing with either alcohol, or ether, the lipoid substances were removed and the mass received by us proved to be inert."

Allen and Doisy then continue and state "in the absence of positive results from the extracts of corpora lutea, Frank's experiments with liquor folliculi might have been considered promising preliminary experiments; as it is we are inclined to believe that their significance was overlooked." If comment is required, it would take the form of stating that I am forced to conclude that Allen and Doisy are not "*inclined*" but "*determined*" to believe that I overlooked the significance of my discovery.

I quote only one more reference to my work by one of these same authors (Allen in Pratt and Allen, 378).

> "In 1922, Frank announced in a *single paragraph* in a critical review that injections of fresh liquor folliculi produced uterine growth in young rabbits in which the ovaries were still intact (four cases). *This was published five years after the work was done.* Due credit should be given Frank for these preliminary experiments."

The italics are mine to draw attention to the statements I desire to illuminate.

I am not sure whether I should feel aggrieved or complimented that my brevity is condemned, because I "*announced in a single paragraph in a critical review*" that fresh liquor folliculi produced uterine growth.

Presumably, therefore, for the same reason my work (141), published in 1917, on freeing the active substance from cholesterol should likewise be regarded as a preliminary experiment because I devoted *less than one paragraph* to this statement. Allen and Doisy confirmed my work in over one and one-half pages of their publication in 1924 (93). In the same article, in 1917, I condensed the report on the use of the castrate rabbit's breast, as a biological test "of the potency of the extracts" in a mere paragraph of seven lines, although Steinach (431) for example devotes pages to a similar test in the guinea pig some ten years later.

May this condemned brevity not be excusable as a permissible vagary, or as a preferable mode of publication in the state of overcrowding which delays publication in the scientific and medical journals?

In reference to the further statement that *"this was published five years after the work was done,"* the following explanation for the implied dilatoriness is offered.

The reason that such a long time had elapsed between the time of my original experiments and their appearance in print was due entirely to private and personal circumstances which I would not refer to had this question not been raised and emphasized. On the day on which I was leaving for Pittsburgh to open a "Symposium on the glands of internal secretion and their relation to gynecology," (140) at the meeting of the American Gynecological Society, in 1917, I was called into active service in the Army. Immediately after my return from France in 1919 I was obliged to stop all work for reasons of health and was unable to resume my experimental work until the end of the year 1922.

In concluding this analysis, I desire to emphasize that the question of priority interests me far less than the imputation necessarily cast that I, who had been engaged in the study of sex physiology since 1905, *could fail to appreciate the importance of my demonstration of the activity of the follicle fluid.* Repeatedly I had drawn attention to the fact that every known observation pointed to follicular production of the cyclical sex phenomena, which at puberty and thereafter, occurred before the formation of the corpus luteum.

> Mention should be made of the fact that Wintz (473) in 1920 had reported negative results from the use of follicle fluid.
>
> Recently, I came across experiments by Wester (468) who in 1921 injected 30 c.c. of follicle fluid *pro die* for 10 days into goats, with negative results.
>
> These negative results appear due to too small dosage.

Further confirmation (if such were necessary) of the activity of the follicle fluid was supplied by Seaborn and Champy (411) (1923) who, by the injection of the follicle contents of the ovaries of mares in heat, induced heat in rabbits as shown by acceptance of the male. They claimed, as we now know erroneously, that the follicle fluid obtained from the mare, when not in heat, was ineffective.

Courrier (77) in 1924 did the same with sows' follicle fluid in castrated guinea pigs and in 1926 (80) in rabbit castrates.

Tsu-Zong-Yung, 1924 (446) by repeated injections of follicle fluid, obtained congestion and growth changes in the genital tract and breasts of immature female rabbits.

Vintemberger, 1925 (457) by the same mode of stimulation, studied the breast changes resulting in male and female rabbits. He used the follicle fluid of cows. Rapid mammary growth was noted.

Since then innumerable investigators have amplified our knowledge of the phenomena due to the active substance contained within the follicle. (See Chapter VI).

CHAPTER V

TESTS FOR THE FEMALE SEX HORMONE

THE last chapter has dealt with the researches on the female sex hormone up to the point of the introduction of the vaginal smear test of Allen and Doisy. This test, as previously mentioned, marks a tremendous advance in the study of female sex physiology, because of the ease of application and the facility of interpretation. The possibility of preserving the same animal and using it repeatedly, as well as the saving of time, which resulted thereby, proved noteworthy. Previously in most instances, an animal had to be sacrificed for each test. Moreover, the animals used by earlier workers, preferably rabbits and guinea pigs, were both more expensive and more difficult to keep, handle, and feed.

The first investigators were satisfied to arrest castration atrophy. The next succeeded in producing in castrates the appearance of a stage resembling estrus or pseudo-pregnancy. The various tests are described in detail, not only because of their historical interest, but also because some of them are still of utmost importance on account of their accuracy and specificity.

In addition to the test for the growth producing factors of the female sex hormone, another set of tests has been elaborated which attempts to analyze and assay the supposed inhibiting effect of the corpus luteum, as well as its sensitizing properties. This type of test will also be discussed.

ASSAY FOR THE FEMALE SEX HORMONE

1. Uterine Growth

This test is based on the observation that a stable condition of atrophy is arrived at, within a short time after castration, by the uterus, vagina, and breasts. This condition, which I have called the "castrate norm" (Frank and Rosenbloom, 1915) (165), varies with each species, and within limits, even in individuals of the same species, depending on the time of life at which castration is practiced. It has been our custom at the time of castration to excise a portion of the uterine horn which is kept for future reference. After the elapse of one or two weeks, the uterus has reached a degree of involution making it considerably smaller than at the time of castration. It is this final stage which is called the "castrate norm stage."

Injections of potent extracts produce a distinct increase in the size, which includes length and breadth, of uterus. In rabbits and guinea pigs it requires from four to six days to produce a marked change. In rats and mice the changes regularly take place within 48 hours. The resulting changes in macroscopic and microscopic appearance correspond closely to the proestrous, estrous, or pregnancy condition of the uterus, vagina, and breasts, depending almost quantitatively upon the amount of material injected, the number and spacing of the injections, as well as the potency of the material.

The resulting change in size, vascularity, increase in musculature, as well as endometrial changes observed in the test animal are specific for each individual species, but are independent of the source of the female sex hormone. Whether derived from human, cow, mare, sheep, pig, guinea pig, rat, or the organ from which obtained (follicle, corpus luteum, placenta, blood, etc.,) the effect is the same.

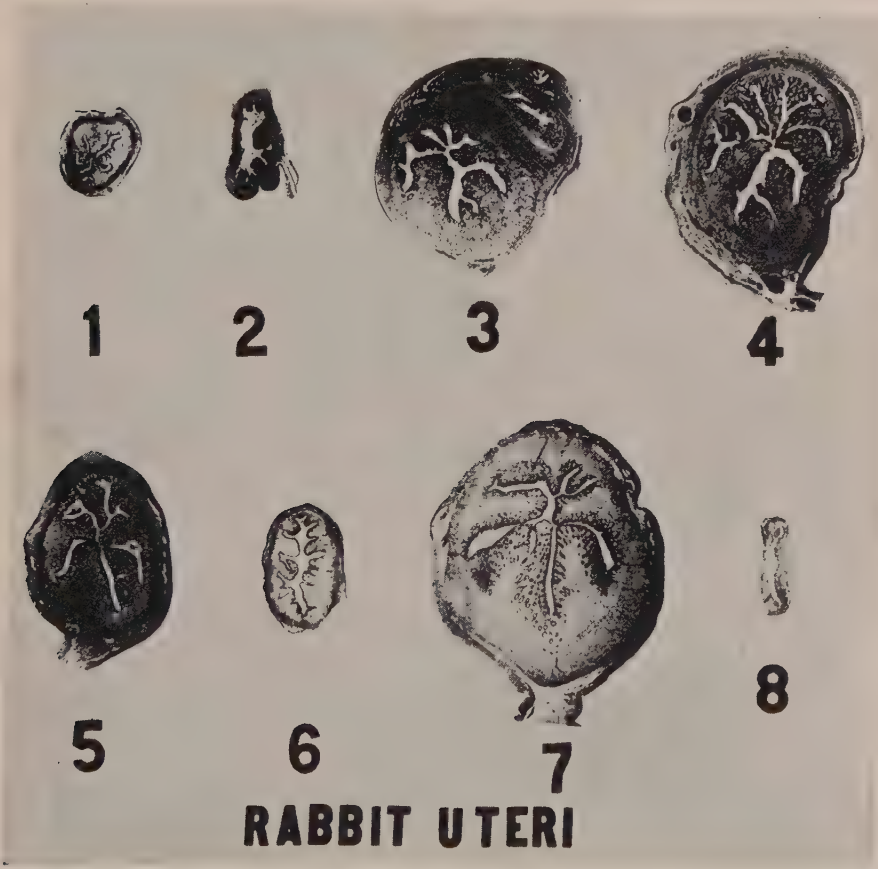

Fig. 30. Cross Section of Rabbits' Uteri.

1. Unoperated control. 2. Castrate control. 3. Castrate after injection of placental extract. 4. Castrate after injection of boiled placental extract. 5. Castrate injected with corpus luteum extract. 6. Horn of uterus removed for control before injection from 5. 7. Castrate injected with placental extract. 8. Castrate injected with insoluble portion of placental extract.

The individual type of gland changes, the number of mitoses, the decidual stroma reaction, the contents of the uterine and vaginal cavity, however, depend upon the animal selected for the experiment. For example, the rabbit's uterine cavity, even when full estrous changes are induced, remains empty while that of the rat or mouse becomes filled with fluid, leucocytes and desquamated cells as is normally the case in this species during estrus.

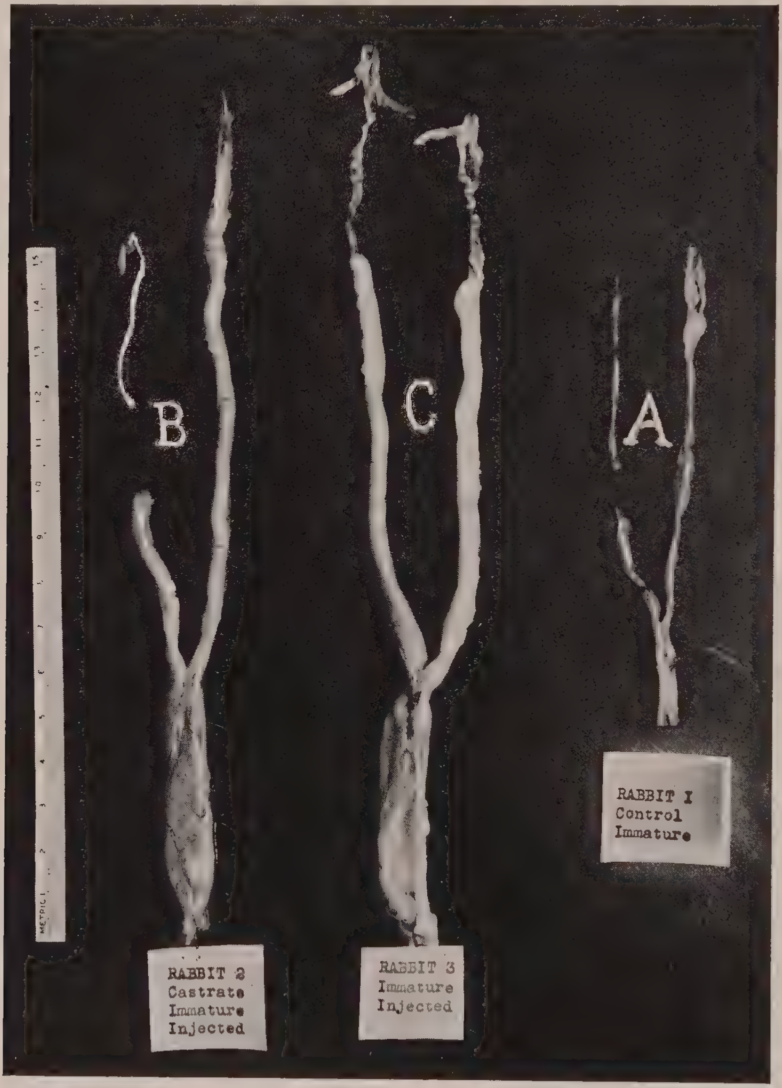

FIG. 31. RABBIT UTERUS TEST.

A. Rabbit 1—Immature control.
B. Rabbit 2.—Immature castrate injected with female sex hormone over period of 7 days.
C. Rabbit 3—Immature, non-castrate injected over same period.

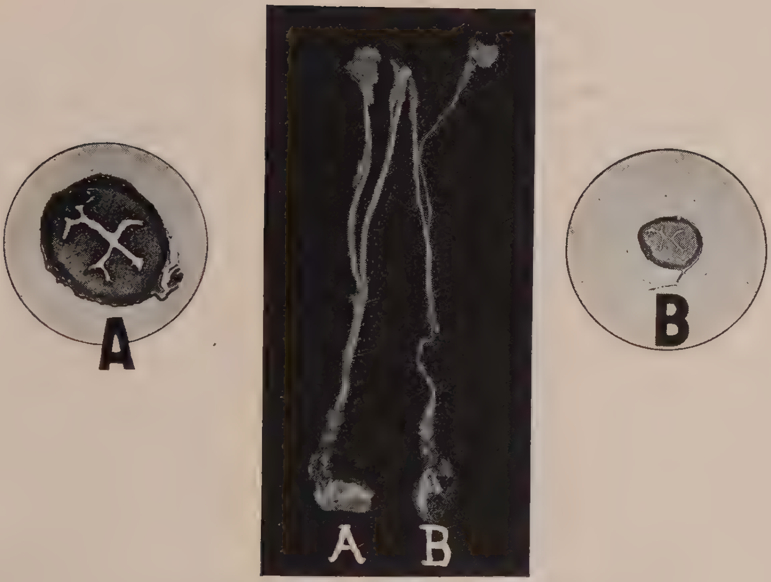

Fig. 32. Rapid Rabbit Uterus Test.

A. Castrate injected with active female sex hormone extract.
B. Castrate control.
To each side of the central figure is the corresponding cross section of the uterus.

Fig. 33. Rats' Uteri.

A. Castrate control.
B. Castrate injected; full estrus due to injection with corpus luteum extract.
C. Castrate in full estrus, injected with placental extract.

Actual experiments have shown me that the tremendous increase in size is not due to a great increase in the percentage of water content of the uterus. My attempt to deduce any useful biological information from this fact has proved futile, nor was I able to make use of the weight of the wet compared to the dried uterus in obtaining constants. (See table).

TABLE

Rabbits treated identically with placental extracts.

Rabbit No.	Body Weight grams	Empty Body Weight grams	Uterus Wet g.	Uterus Dry g.	Per cent Water	Per cent Solids	Uterine Reaction
279	400	340	1.627	0.2435	85.6	17.4	+3
280	480	420	1.495	0.2435	83.7	16.3	+3
281	480	370	1.574	0.2148	86.4	13.6	+3
282	440	362	1.843	0.2750	85.1	14.9	+3
283	495	440	2.100	0.3700	82.4	17.6	+3
284	320	302	1.573	0.3010	81.9	19.1	+3
301	350	312	0.326	0.055	83.1	16.9	control 0
302	325	280	0.331	0.056	83.0	17.0	control 0
303	525	450	0.397	0.061	84.6	15.4	control 0

Standardization

It naturally followed that in performing numerous experiments assayed by means of the increase in size of the uterus, a scale should be devised. Many years of experience convinced me that simple inspection, if carefully carried out, by well-trained observers, and controlled by microscopic cross sections of the uterus, gave the most reliable information. Therefore, in 1917 (139) I reported a scale dependent on the size of the uterus of the rabbit calling:

The castrate normal = 0.
A very slight reaction = +1.
A proestrual reaction = +2.
Corresponding to full rut or estrus = +3.
An early pregnancy or pseudo-pregnancy reaction = +4.

Anything above +4, which was rarely obtained, I classified as +4+ and compared it to the uterus immediately post partum.

The work of Fellner (113-5), Aschner (27), Schickele (401), Frank and Rosenbloom (165), etc. was all recorded by this method. This work has stood the test of time.

By means of this scale I was able to titre extracts with a considerable degree of accuracy, but I have recognized that inexpert or inexperienced observers might make mistakes and record a zero as a weak positive reaction. Uterine growth can also be tested on immature non-castrated animals.

> The use of the ratio between uterus and body weight (weight of uterus compared to the body weight) or even of the exenterated body weight, as employed by Giesy (182) and Schröder and Goerbig (410) and also Brouha and Simonnet (60) are inaccurate. My control experiments convinced me that the variation in body weight, even after the intestines had been removed, was too great to make such a comparison reliable.

2. VULVA

Rabbit. Fellner (113) examined the external genitals of rabbits to determine whether rut was present or not. He likewise applied this criterion to castrates in which extracts had been injected. The signs in rabbits are so slight that this method is of little use.

> Kelly and Papanicolaou, 1927 (249) have recently shown that in the guinea pig the vulvar lips are agglutinated throughout 85 per cent of the cycle and that spontaneous opening of the vulva corresponds to the height of estrus. This observation may be utilized in gauging the potency of extracts if castrates are utilized.

Bitch. Marshall in 1905 (325) as well as Schickele in 1912 (401) watched the vulva of the bitch for rut symptoms both in the castrate and the non-castrate. In the non-castrate, at full estrus, the swelling and bleeding is quite characteristic. Schickele's colored pictures of the effect obtained on castrates are, however, not extremely convincing.

Monkey. Corner (74) as well as Hartman (208) have noted that the sex skin around the perianal region of monkeys shows vivid coloring if sufficiently potent extracts are given.

All these signs are of help in gauging the amount of hormone necessary to produce an effect, but have been largely superceded in those animals in which the vaginal spreads are characteristic, by observation of changes in the smears.

3. BREASTS

The fact that the breasts and nipples increase at puberty has been known since immemorial ages. It was also always known that the breasts and nipples enlarged progressively during pregnancy and contained a cloudy fluid called colostrum. If castration is performed during immaturity, the breasts do not develop, Foges (126). If castration is performed after puberty, regression, especially of the acinar structures takes place, but the larger duct system shows little involution.

As I pointed out as early as 1912 (Frank 136) secretion of colostrum or later of milk is influenced by galactagogues but not by the female sex hormone, and therefore is no index of this hormonal action.

> Lane-Clayton and Starling (262) as previously referred to (page 58) erroneously considered the fetus as the factor influencing the growth and development of the breasts. Bouin and Ancel (53) as early as 1909, in their analysis of the effect of corpus luteum, con-

> sidered breast growth as due to the secretion of the yellow body. Frank and Unger (166) studied the development of the normal rabbit's breasts and came to a similar conclusion. (See Fig. 29, p. 69.)

Rabbit. In 1917 (141) I suggested that growth of the nipple could be recognized by inspection of the rabbit's abdomen, in animals castrated before puberty, and utilized as a sign of the potence of extracts. Personally I always utilized this method in connection with later direct inspection of the size and appearance of the uterus at autopsy, but it enabled me to gauge the necessary dosage as well as the effect produced, with sufficient certainty to select the right time at which an animal should be sacrificed. (See Fig. 27, p. 67 and Fig. 29, p. 69.)

Guinea pigs. Steinach (429) has watched the size of the nipples and the presence or absence of secretion in the breasts of guinea pigs, both male and female, in order to judge whether ovarian transplants had "taken" and were growing. Whether he is justified in considering mammary increase of nipples and breasts in males as a sign of "femininization" is questionable, but need not be discussed in this connection. Recently (1925) he (Steinach *et alia*, 431) uses the breast changes of albino guinea pigs castrated in infancy, as a biological test much as I had done in 1917.

Opossum. Hartman (209) has used the breast in the castrated opossum as an index. As the nipples and breast tissue are concentrated in the marsupial pouch, a positive reaction, according to this author, is very striking and readily recognized.

Allen (5), too has recognized the value of the breast changes in the study of the effect of the female sex hormone, when using monkeys.

4. Premature Puberty

Rabbits and Guinea Pigs. Aschner (27), Fellner (113), Frank and Rosenbloom (165) between 1912 and 1915 regarded the induction of premature puberty in castrated rabbits and guinea pigs as a sign of potency of the extracts employed.

Later (1917) (139), after having convinced myself by innumerable experiments that the active extracts in no way affected the ovaries, a contention that has been sustained by all further investigation of the female sex hormone, in order to reduce the tremendous labor involved in castration of animals which had to be sacrificed, I contented myself, as a routine, to use immature rabbits with ovaries *in situ.* By selecting animals whose weight remained under 400 to 800 grammes, all error from spontaneous occurrence of puberty was avoided. The difference in the gross appearance of the immature rabbit's uterus (control) and the rabbit's uterus in which experimental hyperplasia has been induced, is striking and unmistakable. I may mention that the results which I obtained by means of the rabbit test, and this includes a large amount of chemical research which has never been reported in detail, have been fully substantiated by me and others, by means of other tests, including the vaginal smear test of Allen and Doisy.

Rats and Mice. Allen and Doisy (6) produced premature puberty by injecting follicle fluid into immature rats. In these rodents puberty is dramatically marked by the opening of the previously closed vagina, followed shortly by the appearance of a positive vaginal smear.

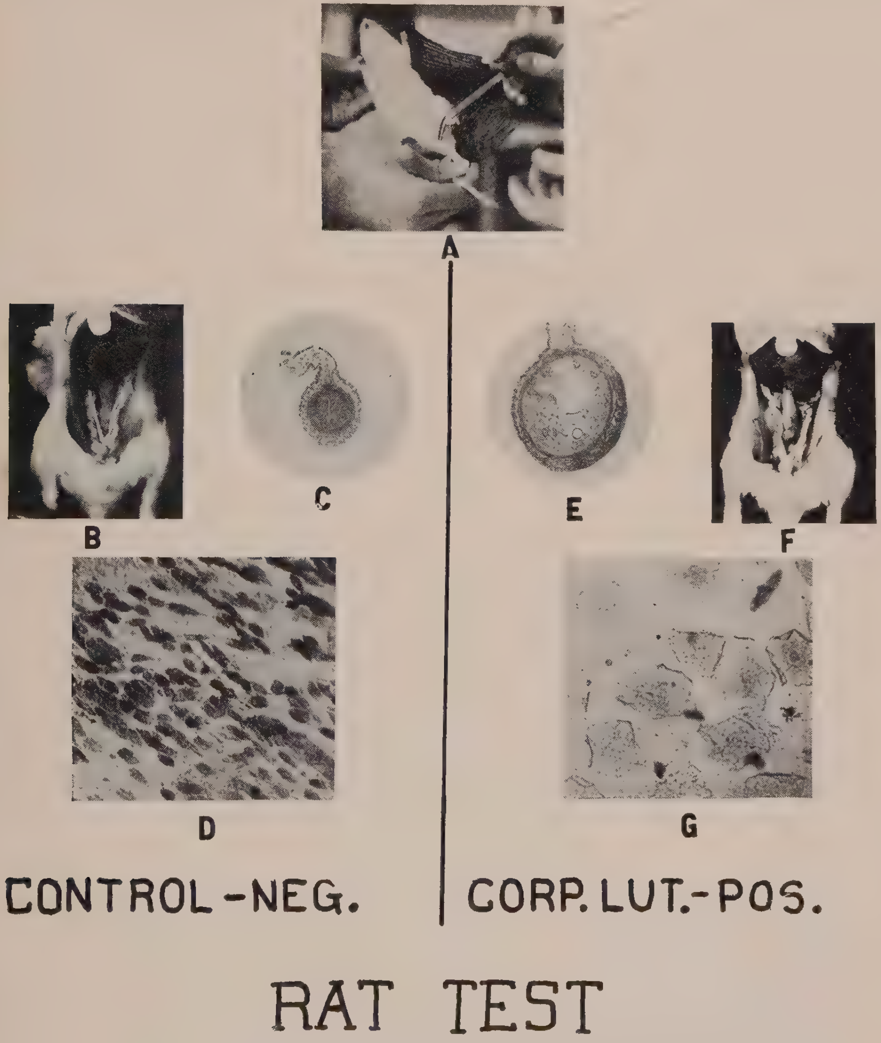

Fig. 34. The Rat Vaginal Smear Test.

A. Method of obtaining vaginal smear from rat vagina by means of glass pipette and suction bulb. B. Appearance of non-stimulated rat's uterus. C. Cross section of uterus. D. Negative vaginal smear. E. Cross section of castrate rat's uterus artificially stimulated. F. Gross appearance of castrated rat's uterus in artificial estrus. G. Positive vaginal smear of stimulated rat.

5. VAGINAL SPREAD TESTS OF ALLEN AND DOISY

Morau (342) described the vaginal cycle in the mouse. Retterer (386) described the changes in the rabbit and guinea pig (1892), as did Königstein, 1907 (256). Stockard and Papanicolaou (433) in 1917 observed and recorded the change in vaginal contents of the guinea pig. Long and Evans in 1922 (320) described it in the rat.

It remained for Allen and Doisy in 1923 (6) to apply this very striking cyclical change of the rodent vagina as a test for the potency, in other words, for the assay and titerage of the female sex hormone.

In either immature or castrated rats or mice, the injection of a potent extract is followed in 48 hours by the appearance of full estrus noticeable in the uterus and vagina. The uterine wall becomes thin, in consequence of a marked distention of the lumen by fluid. The vaginal walls have thickened and are cornified. They then desquamate.

In consequence of this cyclical reaction, marked changes in the vaginal spread of the rat and mouse can be noted. The vaginal content of the castrate, untreated mouse or rat consists of a large number of leucocytes, an occasional eliptical, epithelial cell, and a varying amount of mucus. If such a castrate, in the resting state, is injected, and according to Allen and Doisy the substance to be tested should be injected at four hour intervals for three doses, a gradual change in the cellular composition of the vaginal spread appears. After 24 to 36 hours an increased number of epithelial cells, particularly of the small variety, is noted. After the lapse of 48 hours, a full reaction documents itself by the complete disappearance of leucocytes. Instead of them, the entire vaginal spread is composed of masses of non-nucleated squamous scales. A less complete reaction shows itself in the form of complete absence of leucocytes but a large number of nucleated small epithelial cells amid the squamous scales.

Rat Unit. Allen and Doisy have called the amount of potent substance necessary to produce a full change from the negative to the estrual vaginal spread in a castrate rat of approximately 140 grams (±20 grams) in weight, *a rat unit* (R. U.).

Bugbee and Simond, 1926 (64) have tested the effect of using larger or smaller rats for titration. They concluded that the following formula will obviate inaccuracies due to the effect of direct proportion of dosage to weight.

$$\text{R. U. per c.c.} = \frac{W}{140\,Q}$$

in which W = Weight of rat in grams
Q = minimal c.c. (or g.) which will produce estrus

End Reaction. Many discrepancies arise from the fact that different investigators select different criteria as evidence of a positive reaction. Loewe (310, 312) is satisfied with a percentage count; Frank and Goldberger (157) have a scale from 0–4, anything above 2+ counting as a positive reaction (see p. 184); Laqueur (265) demands a reduction of leucocytes to a very few with at least equal presence of nucleated and non-nucleated epithelium; Allen and Doisy (93) full cornification.

Duration of Reaction. One error arises from giving too large a dose. A full positive will then be obtained quickly—in 18 hours instead of 48 hours—and the positive reaction will continue for several days. A unit dose will give a positive reaction in 48 hours and will begin to decline, as shown by the invasion of leucocytes, in 6–8 hours.

We agree with Allen and Doisy that three injections (aliquot parts) be distributed over one day. Some have given the entire material in one dose (Coward and Burn, 84), others in 3 doses at 12 hour intervals (Biedl, 43). Such variations affect the outcome.

Mouse Unit. European investigators, who prefer to use mice for their work, call the amount necessary to produce the estrual changes in the castrate mouse, *a mouse unit* (M.U.).

Throughout this monograph these abbreviations will be employed. In addition R.U.Kg. and M.U.Kg. will signify rat and mouse units per kilogram and R.U.L. or M.U.L., rat or mouse unit per litre.

Partial Reactions

Loewe of Dorpat, 1926 (312) has utilized the percentage content of leucocytes in comparison to nucleated cells and non-nucleated scales, as an intermediate reaction. In my opinion, such percentage calculation is inaccurate and may lead to mistakes in titration by giving a false impression of great delicacy and accuracy.

In our own work (Frank and Goldberger, 157), we have preferred to subdivide the reaction, as read by means of the vaginal smear, on a scale of 0 to 4, realizing fully that this is a rough and ready method. (Fig. 35).

TABLE

0 = untreated castrate; leucocytes, mucus, a few nucleated epithelial cells.
1 = negative; predominance of leucocytes; increase in nucleated epithelial cells.
2 = still negative; presence of numerous leucocytes; predominance of nucleated epithelial cells.
2+ = weak positive; occasional leucocyte; nucleated epithelial cells.
3 = threshold reaction; no leucocytes; nucleated epithelial cells.
4 = strong reaction; epithelial non-nucleated scales exclusively.

Titration. According to those laboratories in which accurate titration is attempted, especially according to Laqueur (263), it is necessary to utilize at least 5 to 10 rats or mice with a positive percentage result of 75 per cent in order to titre accurately. This is to offset the difference in susceptibility of individual animals.

Method of Obtaining the Vaginal Spread

The technic by which the vaginal smear is obtained is of considerable importance. Many employ a small cotton swab (Loewe, the cotton wrapped around the head of a pin), others a platinum loop.

We utilize a small bent glass tube drawn to a fine tip which is fire-polished (Fig. 34A). By means of a rubber bulb a drop of water is aspirated and, after introduction of the tip into the vaginal canal, the water is repeatedly injected and reaspirated in order to obtain a uniform sample of the vaginal contents. (Also Fig. 49, p. 186.)

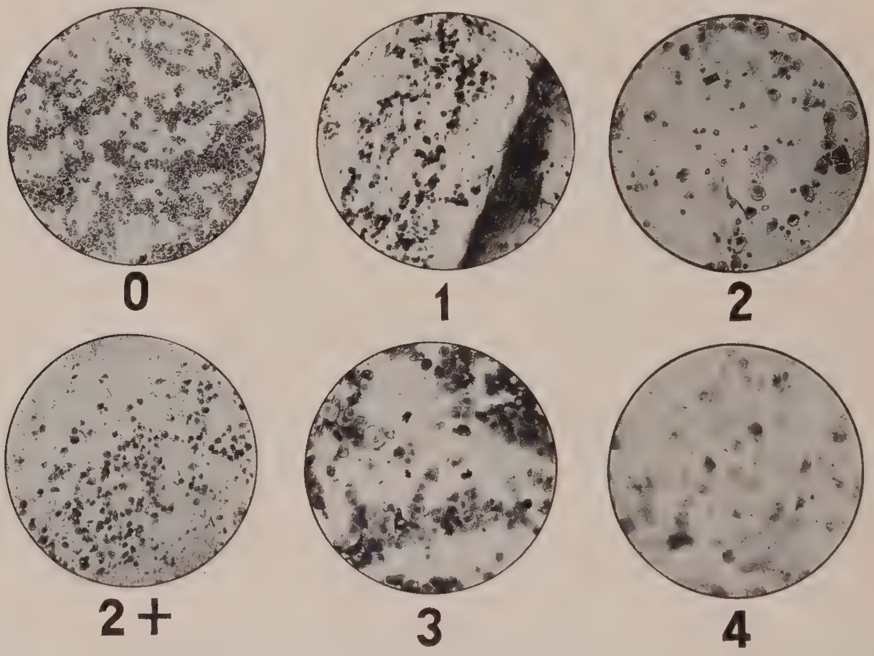

FIG. 35. PHOTOMICROGRAPH OF VAGINAL SPREADS OF CASTRATED RAT.

0—pure leucocytes—(negative). 1—mucus, predominating leucocytes, numerous nucleated epithelial cells—(negative). 2—predominance of nucleated epithelial cells, numerous leucocytes—(negative). 2+—predominance of nucleated epithelial cells, occasional leucocyte—(weak positive). 3—Exclusively nucleated epithelial cells—(threshold reaction). 4—Exclusively non-nucleated squamous scales—(strong positive).

A cannula is used only on one animal. The used cannulae are cleaned by soaking in lye and washed before re-using.

Even under these conditions, unless due care is exercised, the sample may be misleading, being positive in one area and negative in another (See fig. 36).

> Hartman (208) in the monkey, lavages the vagina with 2 or 3 c.c. of physiologic salt solution and allows the washings to settle in a graduated tube. The amount of sediment is plotted.

Accuracy of the Vaginal Spread Test: Precautions

Bugbee and Simond (64) attempted standardization by means of the vaginal spread of rats. Their formula for the rat is described on page 88. This is a convenient but not strictly accurate method. If real accuracy in titration is attempted, Laqueur's method of employing numerous animals is preferable.

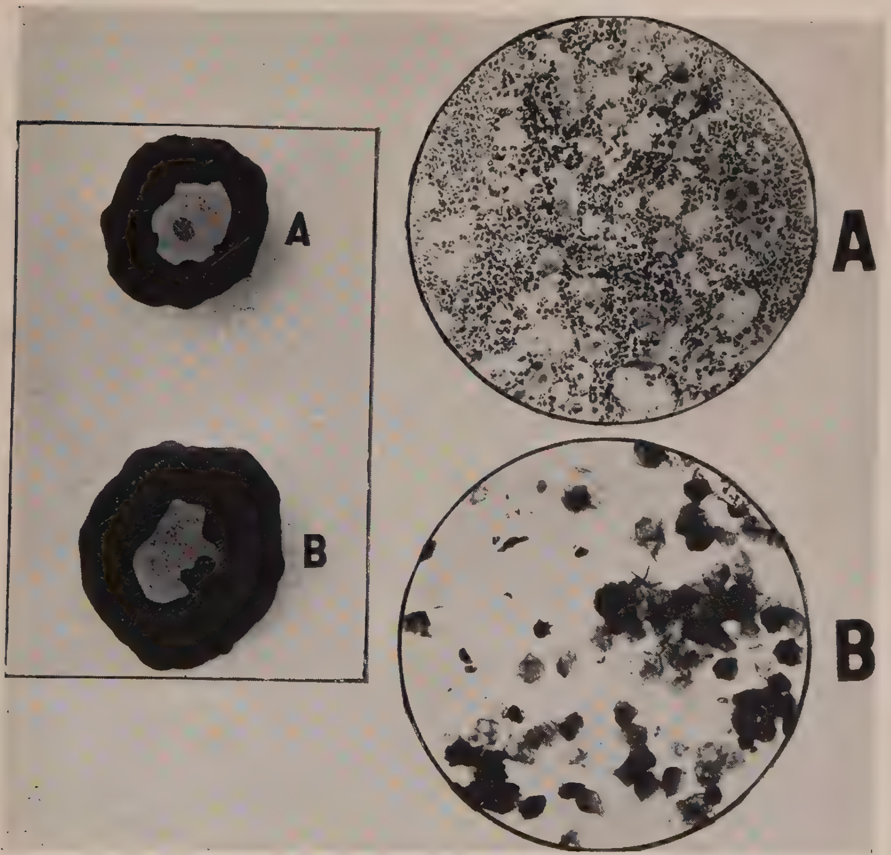

FIG. 36. MISLEADING SPREAD.

To left, very low power photomicrograph of glass slide with 2 areas ringed. These are derived from a single spread from the same rat. A—contains many leucocytes and also nucleated epithelial cells. B—represents a pure 4. This shows how cursory examination of a few fields may mislead.

For titration Laqueur (263) first insisted on the use of 12 (preferably 24) "well castrated" (i.e., observed 4 weeks post castration) mice. Of these at least 75 per cent must react "positive."

Now Laqueur (264) demands the following for accurate titration.

> "An accurate titration," he says, "requires the following:
>
> "At least 12, preferably 24 "well castrated" mice, i.e. animals whose spread has remained fully negative for four weeks, are injected in two series; (a) containing the supposed unit; (b) containing 33 per cent less than the supposed unit.
>
> "Each test is given in two divisions, i.e. to half the mice employed, in order to exclude the so-called possible "day factor." Each dose is divided into 6 parts, injected over a period of 48 hours (each injection consisting of 0.1 c.c. fluid). Of each animal six vaginal smears are taken and examined on the morning and evening of the 3rd and 4th day and many microscopic fields in each slide examined.
>
> "The dose (a) contains the unit if three-fourths of the first series of animals show the positive reaction and of the second batch, injected with 33 per cent less (i.e. two-thirds of the dose "a") less than two-thirds of the smears are positive."

Kahnt, L. C. and Doisy, E. A. (247a) after this manuscript was completed, have published an article on the vaginal smear method of assay of the ovarian hormone, which complicates the test to such a degree as to make it practically impossible to carry it out as a routine measure. In briefest outline, they propose that:

(1) Daily smears be made for 2 or 3 weeks before ovariectomy, only animals with normal cycles to be selected for the operation.

(2) Daily smears for 2 weeks after operation, discarding any animals showing estrous or pro-estrous smears later than the second day after operation. This is the custom of most investigators at present.

(3) Prime the animals with 2 R. U.

(4) One week later test the reaction of each animal by injection of 1.3 R. U. If the smears are negative, discard the animal.

(5) A week later test the reaction to 0. 70 R. U. If positive smears occur, discard the animals.

(6) Use each animal for no longer than 4 months.

(7) In attempting accurate assays, prime all animals by injecting 1.5 R. U., if the reaction of the preceding week was negative.

(8) Use a sufficient number of animals. If 75 per cent of the animals injected with the same volume give a plus reaction, consider that the amount injected contained 1. R. U.

If all of these propositions and recommendations are carried out, the test becomes too cumbersome to permit of work in the average laboratory.

Accuracy of Method

In contrast to this work, the work of Coward and Burn (84) must be placed. These authors speak of 1000 per cent error.

> These authors claim that the result is the same whether 1 or a subdivision into 3 subcutaneous, or 1 intraperitoneal injection be given. According to their test, in 90 rats, all given intraperitoneally in 1 dose, the following results were obtained:

2.5	mg.	estrus	in	7	per cent
5.0	"	"	"	32	"
7.5	"	"	"	31	"
10.0	"	"	"	40–44	"
12.5	"	"	"	55	"
17.5	"	"	"	76	"

I am fully cognizant of the variation in susceptibility of individual animals and that, in common with many biological tests, the Allen and Doisy reaction must be regarded as mainly qualitative. Yet accuracy of technic and care to maintain minutiae unvaryingly, will reduce the variations to a suprising degree. Of this I have convinced myself in our work with blood in which but a single animal can be used for each test.

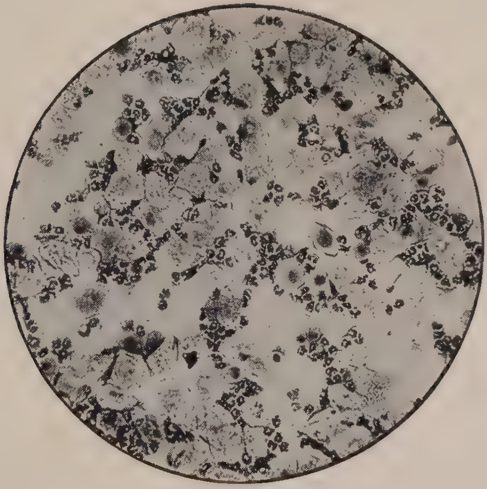

FIG. 37. RAT VAGINAL SMEAR SHOWING DECLINING STAGE.

Twelve hours previously this was a 4. A profuse invasion of leucocytes marks the declining phase of the artificially induced reaction.

> In carefully analyzing the work of Coward and Burn, the following criticism appears warranted. One hundred *unstained* vaginal spreads were examined per hour by two workers. This signifies the examination of 50 spreads in 60 minutes or one and one-sixth minute per spread. In the first place the unstained spread is much more difficult to read and more frequent errors in evaluation are bound to occur. Secondly, in spite of the thousands of spreads that my co-workers and I have examined in the course of years, we find that a much longer period of time is required to examine a sufficient number of fields in a stained, not to mention an unstained spread. Thirdly, the lack of dilution of the material injected for titration, necessitated the injection of minute quantities such as 0.2 c.c. in many instances, which, in my opinion, favors inaccuracy. It is far safer to dilute the

material sufficiently in order that the error resulting from slight variations is reduced to a minimum.

Furthermore, these workers, in judging estrus by the absence of leucocytes, and the presence of cornified cells, classified what we would consider +3 and +4 exactly alike. Finally, in my opinion, intraperitoneal injection, particularly of readily absorbed follicle fluid, is apt to give less accurate results than if the subcutaneous route were used. Subcutaneous injections are more gradually and evenly taken up, therefore giving a more uniform and prolonged effect.

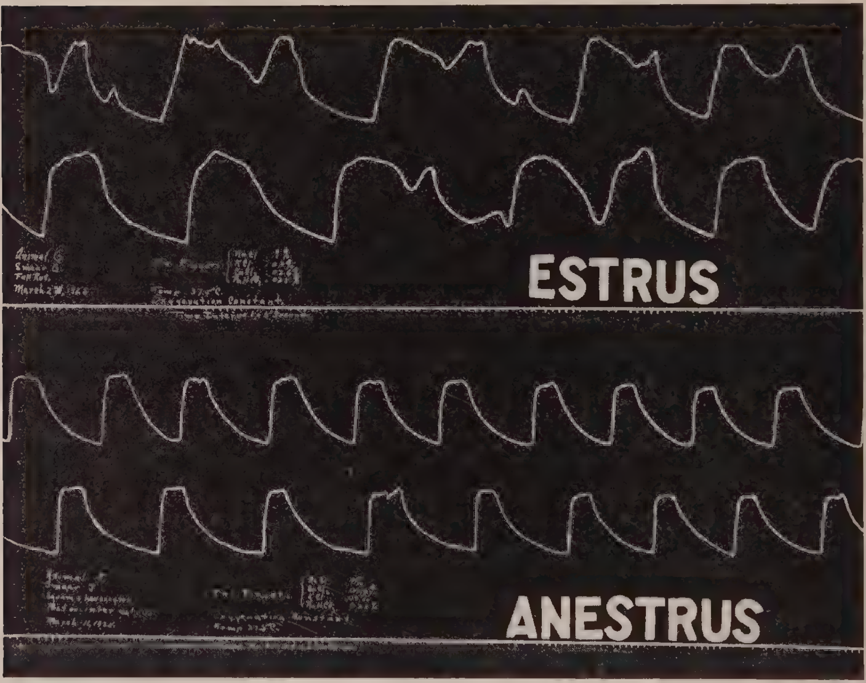

Fig. 38. Kimographic Record of Spontaneous Contraction Rate of Isolated Rat's Uterus.

Above, rat *G* in full oestrus (spread pure squamous epithelium), rate slow, large amplitude. Below, rat *J*, immediate post-oestrus (Anestrous) (spread shows squamous epithelium and leucocytes). Rate rapid, amplitude less.

On the whole I am more in accord with Laqueur (271), who, using 12 animals, if 75 per cent are positives, gauges the accuracy of the method within 15–20 per cent ±.

Errors resulting from reformation of ovarian tissue adhering to the bursa ovarii of rats and more particularly of mice, must be carefully guarded against and kept in mind when positive results occur. Davenport (88) reported that there was the reformation of ovarian tissue after apparently careful castration performed on mice in approximately 22 per cent. Laqueur (269) and others have noted this same reformation with subsequent spontaneous recurrence of estrus. Loewe (312) has suggested that mice, after castration, should be watched for 44 days before using them for injection and assay, in order to avoid errors from this source, as spontaneous estrus always documents itself before the elapse of 6 weeks. In our work, by removing a large area of the peritoneum and Fallopian tubes together with the ovaries, reformation of ovarian tissue has become a very rare occurrence. However, if a positive reaction is obtained, vaginal spreads of such mice or rats are followed for a period of 10 days in all experimental work, in order to exclude even the small possibility of spontaneous estrus.

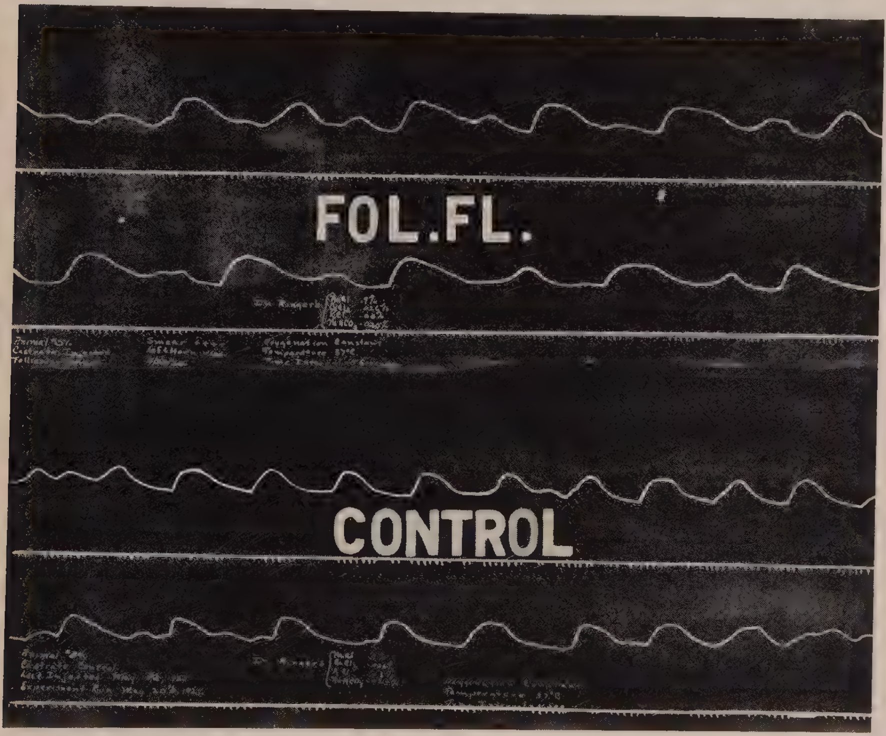

Fig. 39. Kimographic Record of Spontaneous Contraction Rate of Isolated Rat's Uterus.

Above, rat 51, castrate, injected with follicle extract; (spread positive), rate 123.5 seconds. Below, rat 34, castrate norm.; (control) (spread negative), rate 65.3.

6. Rate of Contraction of the Isolated Rat's Uterus

Blair, 1923 (48), Corner (73), Wislocki and Guttmacher 1924, (475), and Keye (250) found that the isolated uterus of rodents and other animals, if placed in warmed oxygenated Ringer's or Locke's solution, contracted rhythmically after the first shock had passed. Furthermore, they noted that the contraction rate varied according to the time of the estrual cycle in which the animal was examined.

Based on this discovery, Frank, Bonham, and Gustavson (153) determined that the rate of contraction of the untreated castrated rat's uterus averaged 1 contraction in 65 seconds. The rate of contraction of a castrate, previously injected and brought to the stage of full estrus, was found to be nearly double as long; 1 contraction in 100–125 seconds. This test was found to be very delicate, the presence of even a few leucocytes in the vaginal spread coinciding with a marked difference in the contraction rate which, then returned to the rapid rate of post estrus.

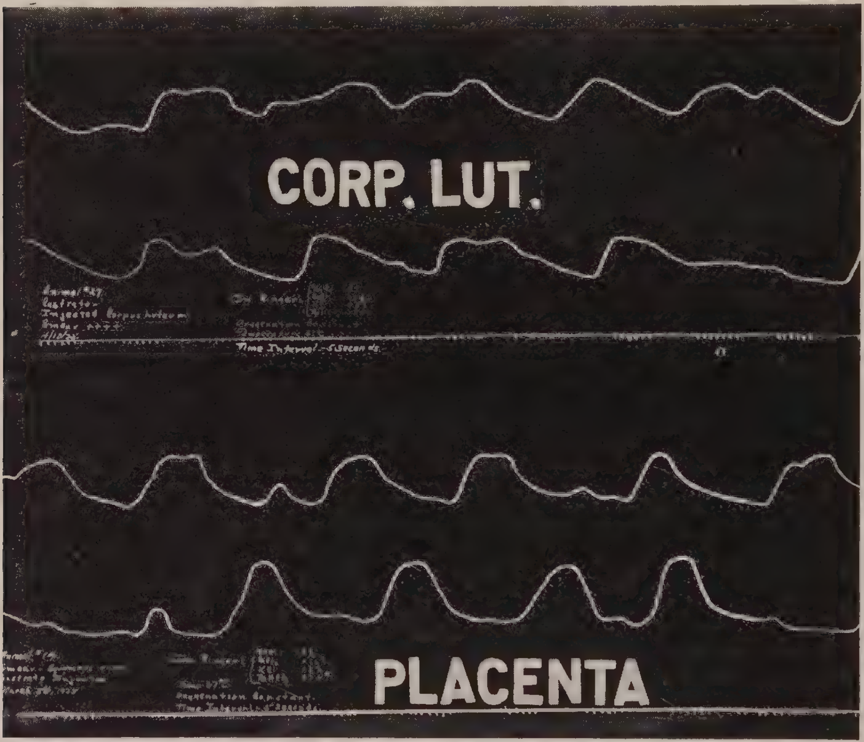

Fig. 40. Kimographic Record of Spontaneous Contraction Rate of Isolated Rat's Uterus.

Above, rat 27, castrate injected corpus luteum; (spread positive), rate 116.4. Below, rat 14, castrate injected with placenta; (spread positive), rate 106.75.

(Figs. 38, 39, 40 from Frank, Bonham and Gustavson, Am. Jour. Physiol., 1925, 74: 395.)

> Seckinger (412) who attempted to utilize the untreated castrated rat's uterus in Locke's solution and add dilutions of the substance to be examined, is employing an entirely different principle which does

> not in any way show the content in female sex hormone of an extract to be examined.
>
> The same criticism applies to the reports of Brouha and Simonnet (58). Fellner (113) used this technic in 1912 before it was generally recognized that every tissue contained some of the pressor principle. (See Matsumoto and Macht, 1919, 329).

Although our reaction, based on the contraction rate, is reliable and delicate, it necessitates the sacrifice of each animal and is of use mainly in the determination of minute variations in strength. It may of course also be used in determining, by an optional method, whether a given extract should be classified as containing female sex hormone, or not.

7. The Chromophil Reaction

Blotevogel (49–50) and later Dohrn and Poll (97) described a method for which they claim extreme, even mathematical accuracy in quantitative assay. This reaction is based upon the discovery by Blotevogel, that the number of chromophil cells (that is cells reacting to chromic acid stain) contained in the Frankenhäuser ganglion (para-cervical ganglion) of the mouse, is dependent upon the stage of the cycle present in the genital tract.

In the immature mouse or in the adult which is in the resting stage (anestrous), the percentage of chromophil cells is small. A progressive increase in the number of these cells occurs toward the approach of estrus. A corresponding diminution follows the attainment of estrus and return to anestrus. A still more marked and progressivelyincreasing accumulation of chromophil cells occurs throughout pregnancy, up to the time of labor. Upon a percentage basis, this increase between the resting and full pregnancy changes, has been found as two and one-half to fifteen and one-half per cent.

To perform the test, castrated mice are employed and are injected with the extract to be examined. After sufficient time has elapsed, the genitals are removed and the utero-cervical region is fixed in bichromate of potassium and then cut in serial section. The sections are projected on paper and an actual count of the number of cells per given area is made. The results are plotted and lend themselves readily to calculation.

This test has the disadvantage of being time-consuming, requiring some 10 to 12 days. It demands especially good technical assistance and accuracy of execution.

The rationale of the test, according to Dr. Poll, whom I was privileged to hear lecture on this subject, depends on the amount of adrenalin secreted. The chromophil staining of the ganglion cells in question is said to depend upon the adrenal reaction. Therefore the possibility of error resulting from the interrelation of the adrenal system and the sex system has to be taken into account. Moreover, some uncertainty obtains as to whether the chrome-staining can be assumed to be an accurate gauge of adrenal activity. Therefore I would caution against the use of this method until additional trial has vouched for its reliability.

8. Use of the Lower Species

Laqueur (264) has found that on tadpoles kept under identical conditions, the addition of potent extracts of female sex hormone to be examined, produces rapid increase of growth. Estes and Burge (108) claim that solutions of paramecium, kept under the same conditions, to which dried ovarian substance has been added, show an increased rate of metabolism. As I have so universally found such ovarian substances inert, and as the effect of the addition of other organ extracts has not been tried for control these experiments will require much confirmation before I am willing to accept them as a specific test.

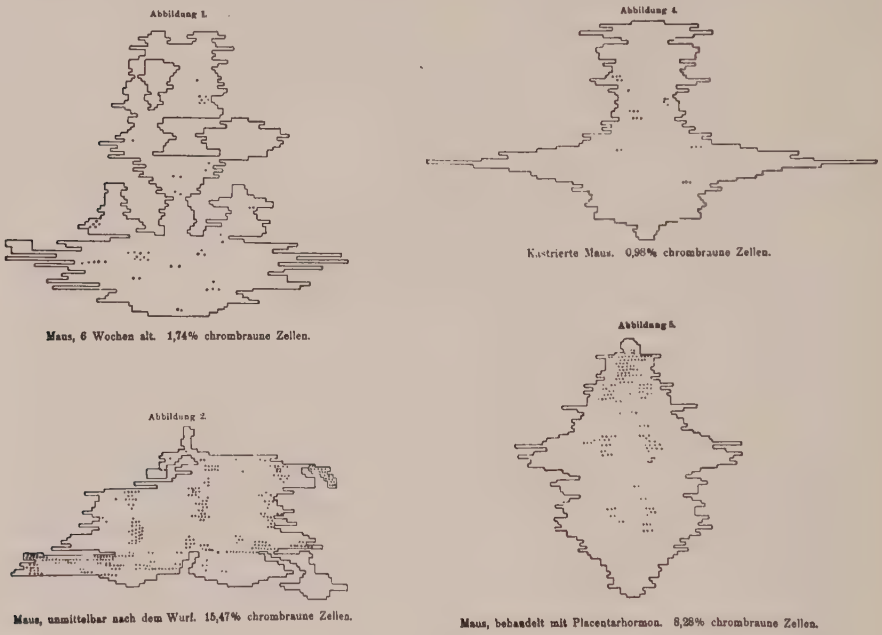
Abbildung 1.
Maus, 6 Wochen alt. 1,74% chrombraune Zellen.
Abbildung 4.
Kastrierte Maus. 0,98% chrombraune Zellen.
Abbildung 2.
Maus, unmittelbar nach dem Wurf. 15,47% chrombraune Zellen.
Abbildung 5.
Maus, behandelt mit Placentarhormon. 8,28% chrombraune Zellen.

Fig. 41. Charts of Chromophyl Reaction.

Fig. 1 represents the plotting of a 6 weeks old mouse with 1.74% chrome brown cells.
Fig. 2, from mouse immediately post partum containing 15.47% chrome brown cells.
Fig. 4, from a castrated mouse contains 0.98% chrome brown cells.
Fig. 5 is plotted from a mouse treated with placental hormone, containing 8.28% chrome brown cells.
(From Blotevogel, Dohrn and Poll, Med. Klin., 1926, 22^2: 1328.)

9. Other Tests Described

Activity. Slonaker (417) has found that the activity of rats measured by their running on an endless chain, is largely influenced by the time of the cycle. Toward the appearance of estrus, a marked increase of activity and energy expended is noticed.

Based on this observation Durrant (103) has tested the activity of castrates to which desiccated ovarian extracts have been fed and has noticed that this activity is increased.

The same caution which I voiced in regard to the experiments on paramecium apply to this form of assay.

Copulation. Allen and Doisy (6) have laid much stress upon the fact that castrated rats, injected with follicle fluid, frequently showed signs of sex excitement and accepted the male.

I have (Frank, 135) pointed out that certainly in the rabbit, the acceptance of the male cannot be regarded as proof positive that the animal is in estrus. It was found repeatedly that pregnant rabbits submit to an active male, and that if a pregnancy was not far advanced, abortion frequently resulted therefrom. Hammond (203) has confirmed my observations.

Therefore, copulation should be regarded with extreme reserve, as a sign of full estrus. I have referred to this point on page 125 in reference to the work of Parkes, who used vasectomized bucks in determining the presence of estrus in X-rayed female mice.

ASSAY FOR THE INHIBITING AND SENSITIZING HORMONE

As previously mentioned, Prenant (379), Beard (37), Sandes (399), and Loeb (298) all believed that the corpus luteum secreted an inhibiting hormone.

Loeb (300) by excision of corpora lutea, shortened the cycle in the guinea pig and in pregnant guinea pigs induced ovulation (303). From this he deduced that the corpus luteum secretion prolongs the cycle by inhibiting the growth and maturation of follicles.

Papanicolaou (356) has, in the guinea pig, claimed to prolong the cycle by the injection of a lipoid extract of corpus luteum. As no description of the preparation of this extract has ever been given, this work does not lend itself to critical comment.

Parkes and Bellerby (370) have described a lipoid extract obtained from the corpus luteum, which prolongs the interval between cycles of mice whose rhythm has been charted. We (Frank and Goldberger, unpublished) have repeated this work numerous times most carefully on a considerable series of animals, following the technic of the authors in question without being able to confirm their results.

Hisaw (225a) has obtained an aqueous extract of the corpus luteum, the details of which have not been published,* injection of which is followed by loosening of the pubic symphysis in castrated pocket gophers, or of loosening of the pubic ligaments of virgin guinea pigs in full estrus. These results we have been unable to duplicate or confirm satisfactorily, because of the author's failure to describe the mode of preparation of the extracts used.

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* Since completion of this manuscript, sufficient details have been published for us to repeat and confirm the pubic ligament relaxing power of Hisaw's extract.

The chief method of assay as yet available, therefore, consists in prolonging the interval between estrus waves, or temporarily inhibiting estrus during the period of injection, in animals whose periodicity has been previously ascertained. We (Frank and Goldberger, unpublished) have found this method uncertain.

Whether Hisaw's titration by the relaxation of the pubic ligaments is due to the same "inhibiting" factor is impossible to say at present. Parkes and Bellerby's extract is lipoidal, Hisaw's aqueous.

Sensitizing effect. It will be recalled (page 51) that Loeb (298) obtained artificial placentomata or deciduomata which correspond to the maternal portion of the placenta in guinea pigs or rabbits and that Frank (135) in 1911 produced deciduomata in rats post partum. Corner and Warren (75) confirmed Frank's experiments. In 1922 Long and Evans (320), by stimulation of the rat's cervical canal, inhibited estrus sufficiently long to produce artificial deciduomata.

These transient tumors were produced in rats by traumatizing the uterine wall while the corpus luteum was in a flourishing state. Teel (438) prolonged the life and persistence of the corpus luteum by injecting anterior hypophysial extracts and by this means was able to produce deciduomata in rats.

Frank (147)was unable to obtain such artificial tumors in castrated rats or in large series of castrated guinea pigs (unpublished), by means of extracts containing female sex hormone.

Weichert (467a) has recently reported obtaining artificial deciduomata or placentomata in castrated rats in which he first injected follicle fluid. After the elapse of two days, he injected some of Hisaw's corpus luteum extract and then passed sutures through the uterine musculature. The Hisaw fluid was injected for four more days with resultant deciduomata such as have been described by Loeb. If this work is confirmed, I consider it incontrovertible evidence of the sensitizing influence of the corpus luteum and strong evidence in favor *of the secretion of two distinct hormones by the ovary*. The method would also offer a reliable form of assay for the sensitizing factor of corpus luteum secretion.

The work of Corner and Allen (74a) and Allen and Corner (12a), which has appeared since the above was written and which was referred to on page 56, by other methods has definitely shown the sensitizing influence of the corpus luteum secretion. In addition, we (Frank, Gustavson, and Goldberger, unpublished) have obtained both female sex hormone (lipoid fraction) as well as inhibiting-sensitizing (aqueous fraction) from the *same batch of corpus luteum* simultaneously.

For the sake of completeness the Manoilow "Sex Hormone Reaction" is mentioned. Popow (377) for example states that he had only 11 per cent of incorrect results in 71 normal subjects. The significance of the reaction is unknown. It may be affected by the rate of oxidative processes. It appears to have nothing to do with the female sex hormone.

CHAPTER VI

MODERN RESEARCHES ON THE FEMALE SEX HORMONE

IN ATTEMPTING to sift the overwhelming amount of literature dealing with the female sex hormone, which has appeared since 1923, it has become apparent that the main achievement of the last five years has consisted in tracing, by means of the more delicate Allen and Doisy vaginal smear test, the distribution of the active substance throughout the vegetable and animal kingdom. In particular, investigations dealing with the distribution of the female sex hormone in the blood, the urine, and feces have already proved of great importance, and promise to be of much greater value. The second achievement of note has been the preparation of a "water soluble" active fraction. This achievement will be discussed later in connection with the chemistry of the female sex hormone. On the whole, the results of the intensive study of the chemical composition and chemical nature of the hormone have proved sterile and disappointing.

In the period between 1896 and 1923 it was definitely determined that the cyclical phenomena depended upon the presence and the functional activity of the ovary. Castration effects were found to be relieved temporarily by implantation and consequent absorption of hormone, and more permanently by successful auto- or hetero-transplants of ovarian tissue. By means of numerous investigations it was also proved that the follicle fluid, as well as the placenta, contained female sex hormone. In regard to the activity of the corpus luteum, differences of opinion still exist. Parkes and Bellerby (362) believe that the cycle is independent of the ovogenic parts of the ovary. A majority of investigators consider the corpus luteum as one of the active sources of the female sex hormone. Even Allen (Pratt and Allen), 1926 (378) now concedes that the human corpus luteum contains the active substance.

> Parkes and Bellerby, 1926 (366–370) on the other hand, as I have tried to show (page 124), claim that after destruction of all ovogenic tissue by means of the X-ray, the ovary still secretes female sex hormone, and that the cycle, at least in the mouse, continues with no, or very little, change. Their point of view is of such great theoretical importance that I will have to take it up in more detail later.

The work of 27 years which I have thus summarized in a few paragraphs, represents a tremendous amount of labor expended by many workers who were handicapped by the difficulties of testing for and assaying the active substance with the tests then available. However, as will become apparent in this chapter, their work including some of the chemical researches, on the whole, bears the spotlight of the severest criticism, and, with the exception of certain additional findings, has merely been confirmed, and in some instances, amplified by the most recent investigations. Recent investigators, it should be kept in mind, have had the great advantage of being able to use the vaginal spread test of Allen and Doisy which has minimized the time consumed, the labor expended, and the cost involved.

The way for all of this work was paved by earlier researches which clarified the sex cycle in some of the lower animals. This work dates from 1889 on, when Morau (342), Lataste, 1892 (276), as well as Retterer, 1892 (386) and others, including Heape, 1899 (214), determined that regular cyclical changes took place in the sex organs of different species. These researches came to a head with the work of Stockard and Papanicolaou, 1917 (433) and Long and Evans, 1922 (320) which enabled investigators to determine the exact stage of the cycle in the living animal. Finally Allen and Doisy applied this to elaborate the vaginal smear test (6).

Just as the older work aimed to counteract the effects of castration, to study the action of potent extracts, and to determine the site of origin of the potent substance, the newer researches have done likewise. The results may be classified under the main headings of the induction of estrus, pseudo-pregnancy, and premature maturity; the production of the mating instinct in castrates, analysis of the growth curves; prolongation of the cycle; antimasculine effect; *in vitro* experiments, etc.

All of the articles referred to are readily accessible although widely scattered in the literature. I shall therefore attempt little more than to summarize them most briefly for the reader. Those especially interested can look up the minutiae and detail by means of the references given. My main effort is directed rather toward correlating the work of individuals or teams in such a fashion as to give the reader a panoramic view of what has been accomplished, what significance may be attached to the findings, and in what fields new work is most promising.

Induction of Estrus

With Follicle Fluid. At the same time that Allen and Doisy (6), in 1923, published their vaginal smear test, they described the effects produced by injecting follicle fluid and lipoid extracts of follicle fluid. Every stage, from anestrus to estrus, could be reproduced both in the vaginal spread and in the vaginal walls and uterus of castrated rats. They emphasized that corpus luteum extracts gave negative results. Allen 1924 (12) and his group were able to obtain the active substance from the follicles of hens' ovaries and from human corpora lutea, 1925 (11). The same investigators were able to induce estrus in castrated mice as well as to produce menstruation in castrated monkeys, 1927 (5).

Courrier (77) in 1924, by means of follicle extract and follicle fluid, produced full estrus in a guinea pig. In 1926 (80) he reported similar results with castrated rabbits.

Courrier, 1925 (79) likewise showed that the regressive changes in the Fallopian tube of the castrated rabbit were promptly relieved by injection of the female sex hormone.

Hartman, 1926 (209) produced estrus in the castrated opossum by means of follicle fluid and placental extracts.

Truffi, 1927 (445) showed that the uterus of a hibernating animal (*Erinaceus europeus*) responded to follicle fluid.

With Placenta. Although I have in the above experiments stressed the fact that positive results were obtained with follicle fluid, these authors all obtained equally striking results with placental extracts.

With Corpus Luteum. Frank and Gustavson, 1925 (162) reported full estrous effects by means of corpus luteum extracts derived from hogs and cattle. We examined various batches of corpora lutea of different ages and found that at the beginning of the involutionary stage the corpora contained the most female sex hormone (see page 136). This led me to distinguish sharply between the content of the extracted gland and the amount actually secreted by this or any gland of internal secretion. While each cell of the corpus luteum is in close proximity of wide open capillaries, the secretion is poured into the blood stream without storage. Only when the capillaries involute, which takes place before the secretory activity of the corpus luteum has ceased, is there a short and temporary local storage of the active substance.

From our investigation we noted that the follicle secreted female sex hormone to the time of ovulation. From then on, in the fertile cycle, the corpus luteum continued the secretion at least until the placenta took on this essential task. In order to properly designate this continuous relay action of follicle, corpus luteum, and placenta, we called this triad the "*Gestational Gland*." Loewe, (317) as well as Brouha and Simonnet (60), at once accepted this interpretation.

Pratt and Allen, 1926 (378) have obtained female sex hormone in the corpus luteum of the human female.

Zondek and Aschheim, 1925 (483) by means of their implantation method, found that the human corpus luteum, before menstruation, was active; after menstruation, inactive. This is as the theory would have it—menstruation occurring when the corpus luteum no longer secretes the hormone. They found the pregnancy corpus luteum was active for several months of gestation, as far as its female sex hormone content is concerned.

Loewe, 1927 (314) found such corpus luteum extracts as he purchased and prepared inactive.

We (Frank and Gustavson) see no reason to change our stand on this important question, particularly as we took the precaution to test the effect of the corpus luteum extracts we investigated (hog, cow [1925], human [1926]) not only by the Allen and Doisy vaginal smear reaction, but also by means of the uterine estrous and pseudo-pregnancy effects induced in castrated rabbits, as well as by the contraction rate of the isolated rat's uterus, by the method of Frank, Bonham, and Gustavson. All of these tests proved uniformly positive. (See Chapter V.)

Recently we (Frank, Gustavson, and Goldberger, unpublished) have obtained both female sex hormone and sensitizing hormone from the same batch of corpus luteum.

Induction of Premature Puberty

For the results obtained by earlier investigators, see page 14.

Allen and Doisy (6, 8) in 1923, described the induction of premature maturity in rats and mice. In these animals the onset of puberty is strikingly documented by the opening of the vagina, usually followed in a few days by an estrous smear. They found that if the injections with active substance were stopped, these animals had no further cycle until the normal time of puberty arrived.

Frank, Kingery, and Gustavson (164) in 1925, on the other hand, in a number of rats, induced premature puberty and shortly after the cessation of injection, noticed that some of these immature animals continued to have cyclical manifestations until the cycle merged with that of the normal time of puberty. Today we are forced to believe that the spontaneous continuation of estrus may have resulted through the presence of unsuspected anterior lobe pituitary extract, rather than from the female sex hormone (See Zondek and Aschheim, page 14). However, investigation of the ovaries of these immature animals did not demonstrate the hyper-ovulation and luteinization described by the above authors, which is regarded as a pituitary effect.

> The opening of the vagina in rats is certainly not directly due to ovarian influences. We have castrated very small rats at the age of 14 days and found that their vagina opened at the same time as that of their litter mates.

Laqueur in 1927 published an elaborate study on the increase in size and weight of the genitals in rats in which premature puberty had been induced (271). The increase in weight in certain instances reached 300 per cent. He also obtained these effects in guinea pigs, mice, and bitches. This study fully confirms the work of earlier authors.

Time Element in Producing Estrus; and Growth Curve

From the studies of Allen and Doisy, 1923 (6, 93), as well as the work of Courrier, 1926 (81), the former using rats, the latter guinea pigs, it was found that the time required to produce the anabolic changes leading to estrus, in the Müllerian tissues and breasts, was independent of the amount of active substance injected. In my own investigations, I also have found this to hold true within a limited range. Very large amounts, I have observed may, however, bring on full estrus in 18 instead of 48 hours in the rat.

In 1916 (139) I described the growth effects produced as proportionate to the amount of active substance given, until the full growth reaction was attained. Beyond this limit no increase in growth could be obtained by increase in the amount of female sex hormone injected. However, until this limit was reached, the effect was approximately directly proportionate to the amount received. Thereafter the duration and persistence of the reaction depended upon the dosage given and, apparently also upon the rapidity of its excretion.

Laqueur (271) in 1927 has fully confirmed my observations. He found that a smaller dosage (approximately 0.1 the amount) was needed to produce uterine growth, while ten times larger dosage was required to effect full estrous changes. His attempt to analyze this further and to distinguish between growth and estrous phenomena, appears to me fallacious. In my opinion, the growth phenomena may be compared to the early proestrus, the more advanced changes corresponding to estrus. The differences in the results achieved are due to quantitative, not qualitative, difference. "In general," says Laqueur, "the amount of growth effect increases with the dose; exact regularity does not obtain. With very large doses growth cannot be increased beyond a certain degree."

Parkes and Bellerby (367) determined that the amount of active substance, which they called "oestrin", needed to force estrous phenomena upon the pregnant mouse, (which female sex hormone they believe overrides the inhibiting effect of the corpus luteum) increases progressively as pregnancy advances. Their analysis (368) goes even further. During lactation they claim that as much as 10 M.U. are required to produce estrus. Therefore they conclude from the fact that the ovariectomized lactating mouse requires only 2 M.U., that the corpus luteum of lactation likewise inhibits estrus, and that its action must be overcome before estrous phenomena can be forced upon lactating animals.

The *weight factor* of the animal used also comes into question (see p. 88 for details).

Growth due to Injections of Non Specific Substances

Considerable uncertainty has resulted from the publication of experiments, performed by experienced and well known investigators who have reported positive growth phenomena observed on the uterus, due to the injection of non-specific substances. Robinson and Zondek, 1924 (393) investigated numerous biogenic amines as well as adrenalin and histamine. They employed rabbits in their experiments, and utilized the ratio of the wet weight of the uterus to the entire body weight of the rabbit. This form of test, in my opinion, is unsatisfactory, as I was forced to emphasize in connection with the later work of Giesy (see p. 132). I found that the tremendous differences resulting from weighing the animal before or after meals, nullified such ratio. This error could not be avoided when the entire gastrointestinal tract of the animal was removed from cardia to anus, because of the difference in amount of blood contained in the splanchnic area ("exenterate weight," "empty body weight").

Therefore the positive results of Zondek and Robinson with aolan (sterilized milk), adrenalin, and histamine must be accepted with much reserve, particularly as careful and ample repetition of this work, employing the rat vaginal smear technic, as well as inspection and microscopic examination of the uteri of rats and rabbits convinced Gustavson and me, 1926 (163) that none of these substances activated the Müllerian system or produced anabolic changes therein. Levin, 1927 (281) too, obtained no positive reactions with histamin.

According to R. Meyer (338) both Kraul and Hirsh-Hoffmann were able to prolong estrus in normal cyclical mice by means of pilocarpin, in which case unusual growth of follicles was produced, and also with "adrenosan," in which case persistence of corpora lutea occurred. Meyer concludes that vagotropic drugs increase follicle production and sympathicotropic drugs decrease it. The actions result by means of effects produced on the ovaries. These results require further investigation and confirmation as they appear important from the view point of therapy.

Schröder and Goerbig, 1921 (410) reported an increase in the size of the uterus of virgin, immature rabbits, following upon the injection of liver extracts. Until recently I was unable to account for their positive findings, as all of our own experiments with liver extracts have proved negative. However, Gsell-Busse, 1928 (194) has found (as mentioned on page 115) that bile as well as sodium taurocholate appears to contain a large amount of female sex hormone. These results we have amply confirmed (Frank and Goldberger, unpublished). I therefore suspect that the liver extracts of Schröder and Goerbig may have contained enough bile to produce uterine changes.*

Local Growth Effects

By a very graphic experiment Loewe, 1926 (317) was able to demonstrate a growth effect localized to the uterus. In a castrate rat he tied off segments of the uterus on two sides. On one side, as the control, a small quantity of oil was introduced into the isolated loop. On the other side an equal quantity of oil containing female sex hormone in solution was injected. This loop showed marked growth and estrual changes.

Courrier (78) in 1925, found that injection of follicle fluid into the vagina of the castrated guinea pig, led not only to local but also to general anabolic changes which resulted in full estrous phenomena if sufficient of the active substance was injected. This experiment therefore simply demonstrated that the active substance was absorbed through the vagina.

Induction of the Mating Instinct

As early as 1911 I showed (135) that pregnant rabbits will accept a vigorous male. Hammond and Marshall, 1923 (204) have likewise described this aberration. It is for this reason that I have always been unwilling to regard the act of copulation in laboratory animals as an accurate or fully reliable index of the occurrence of estrus, although Bouin and Ancel (52) as early as 1908 have utilized this method for discovering and marking estrus in the rabbit, employing vasectomized bucks. Leo Loeb in 1909 (298) tied the tubes of the female and then permitted copulation. Parkes and Bellerby, 1926 (359) have used the same technic as Bouin and Ancel in the mouse.

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* In restudying my records of experiments performed, I noted with interest a single experiment in 1915 in which a strong positive effect was obtained with human bile (sex unknown). The investigation was not pursued further.

Allen and Doisy, 1923 (6) in their earliest publication emphasized the fact that the follicle extract reactivated the mating instinct of castrated rats. In a later publication, 1924 (9) they state that copulation was noted in 7 of 11 castrates receiving injections. I have found that the active substance, whether obtained from the follicle, the placenta, or the corpus luteum, has an identical effect in this regard. The use of the term "estrous hormone" or "hormone of estrus" is, however, inaccurate as I have previously pointed out (page 76), because estrus is merely one stage in the sex cycle. It is the sex stimulation as a whole, not copulation nor estrus which is induced.

Prolongation of the Cycle

Prolongation of Estrus. Early in our work we discovered that continued injection of sufficient amounts of active substance would maintain the anabolic changes of Müller's tract and of the breasts indefinitely if sufficient material was used. Huge hyperplasia corresponding to immediate post partum conditions resulted throughout the experiment, some of which were continued for 30 days in rabbits, guinea pigs, and rats. (See Fig. 25).

Allen and Doisy (93) maintained rats for at least two weeks in full estrus without appearance of any leucocytes in the spreads. Zondek and Aschheim (485) did likewise.

Laqueur, 1926 (268) was able to keep normal mice in full estrus continuously for six weeks by a daily dose of 2 M.U.

Siegmund (415) on the other hand, was able to keep castrated mice in estrus, but failed with normal cyclical animals as well as with pregnant ones. Apparently the dosage used by him was insufficient.

Prolongation of the Interval (Pseudo-Pregnancy)

It must be remembered that physiologically the interval between cycles is prolonged by coitus in both the mouse and rat even if the fruition of copulation is interfered with by vasectomy of the male (Parkes, 359) (Tuisk, 448). This period is known as pseudo-pregnancy.

The same result, according to Long and Evans (320), can be produced by mechanical irritation of the cervix in rats. This irritation is readily produced, I have found, by means of an electric otoscope inserted in the vagina and a small piece of wire which may then be passed by sight through each of the two cervices, and allowed to rest there for a minute or two. Through what mechanism this stimulus produces prolongation of anestrus has not been determined.

The interval may also be prolonged by indifferent and non-specific factors which interfere with follicle growth. Among these are insufficient food (especially food lacking in vitamines), cold quarters, and disease. The uterus under these conditions is thin, avascular, and atrophic in marked contrast to the uterus during pseudo-pregnancy.

Breast Increase

It will be recalled that all of the earlier investigators, including Aschner (27), Schickele (400–1), Herrmann (217–8), Frank and Rosenbloom (165), obtained increase of the breasts and nipples of immature as well as of castrated animals by means of a potent extract. In 1917 I proposed this increase in the mammillae as a convenient biological test for determining the potency of extracts. These changes, I noted, were very poorly marked in the rat, but were distinct in the rabbit and guinea pig. It is well to remember that as analyzed by Frank and Unger (166), these growth changes occur physiologically in connection with the first estrual phenomena. The growth of the main duct system is permanent, that of the terminal ducts and alveoli, showing a cyclical variation. Failure to take this physiological change into consideration originally misled Lane-Claypon and Starling (262) as I pointed out (see page 58).

Both Tsu-Zong-Yung, 1924 (446), and especially Vintemberger, 1925, (457) studied the stimulating effect of follicle fluid, in particular, on the breasts of rabbits. We have found the results, whether obtained by means of follicle fluid, corpus luteum or placental extracts, identical. Laqueur in 1927 (275) again drew attention to the breast phenomena, employing female and male rats.

Lipschütz (291) as well as Steinach (429) utilized the breast growth in males as an indication of successful "hyperfemininization," which they produced by means of ovarian transplants or injection with female sex hormone. Whether their interpretation is justified is quite another question. It would seem that the breast is an indifferent tissue, a bisexual gland, which, however, responds by anabolic changes to the female sex hormone stimulus.

Hartman (209) has recommended the inspection and palpation of the breasts of the castrated opossum as a biological index of the effect of female sex hormone. In this marsupial, the nipples and mammary glands are concentrated in a portion of the abdominal pouch and therefore readily lend themselves to palpatory examination.

Growth Effects on Lower Forms

Laqueur (271) has determined that if female sex hormone is added to cultures of tadpoles, increase in growth results. Estes and Burge (108) report that if ordinary desiccated ovarian substance is added to a growth of paramecium, increased metabolic activity results, recognizable by the utilization of increased amount of sugar in the substrat and increased multiplication.

We (Frank and Goldberger, unpublished) thought that this difference in growth might be due to the large amount of material added, but on investigation of the desiccated powder used, (Parke, Davis and Co.) found that it contained no appreciable amount of milk sugar or other substance likely to affect the metabolic rate by additional supply of nourishment. Further experiments are needed to confirm the interpretation of Estes and Burge.

Effect on the Basal Metabolism

I have always considered the report of Loewy and Richter, 1899 (319) most interesting. These investigators found that the basal metabolic rate of women was reduced by castration. They reported an increase following the use of desiccated ovarian substance, which made me accept the accuracy of their investigation with much reserve.

In addition to the experimental work on paramecium just mentioned, (Estes and Burge, 108) Zondek and Bernhardt in 1925 (490) employed freshly dried pig's ovaries on a woman castrated two years previously and found that the basal metabolism was raised 12.4 per cent.

A careful study by Geist, Goldberger, Reiss, and Lande (177) in which a large number (48 patients) of women were investigated before and after castration and in whom no effect on the basal metabolism was observed, emphasizes the possibility of experimental error in the human female.

Laqueur, on the other hand, has performed a series of experiments which are convincing in their nature. Laqueur (268, p. 9) made accurate gasometric measurements of the oxygen and carbon dioxide consumption of 6 mice simultaneously. The investigation was pursued daily over two or three weeks, each part of the experiment lasting for three hours. It was found that an increase of basal metabolism resulted from injection of female sex hormone into female castrates. No such effect could be obtained in the male castrate with female sex hormone.

Apparently, therefore, no further doubt need be entertained that castration, on the one hand, is uniformly followed by a slight diminution in basal metabolism, and that, on the other hand, the injection of female sex hormone again raises the basal exchange to a normal level, and that this effect is sex specific. (See also Part II, page 266).

Effect of the Male Sex Hormone on the Female Sex Organs

Little is known of the male hormone largely because accurate methods of testing for its presence or effect are wanting.

Such roundabout methods as the implantation of cocks combs into a test animal, with resulting growth or atrophy (depending upon the presence or the absence of the hormone) have been practised by Carideroit and Pézard, 1926 (67) and Busquet, 1927 (66).

McGee, 1927 (333) used capons, whose comb development was regarded as an index of the strength of the male substance injected.

> This substance was prepared from bull's testis by the technic of Frank and Gustavson, 1925 (162).
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> Both Moore of Chicago, (340) and Loewe of Dorpat (309) have written of a test based upon the length of survival of motility of the spermatozoa in the epididymis deprived of testis, but have not described the technic. If an active male hormone is injected the sperma remains alive for a longer period.
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> Funk and Harrow, 1929 (171) were able to prevent atrophy of the comb in cockerels by the injection of the urine of men.

Fellner (115) in 1921 claimed that uterine growth is stimulated in castrated rabbits by the injection of extracts obtained from the testis. Any observation published by such an experienced and meritorious pioneer as Fellner should be received with the utmost respect, and it will require much negative evidence before I am willing to disregard his observation.

Carminati (70) reports a positive vaginal smear reaction obtained with rats testes in female castrate rats. We (Frank and Goldberger, 160–161) have repeated and amplified his experiments with uniformly negative results. Laqueur (264–274) reports a positive reaction with his testis extract.

All the extracts prepared by Gustavson and myself (Frank and Gustavson, 162) as well as by Frank, Goldberger, and McGee (161) have proved negative in producing enlargement of the castrated rabbit's uterus or in affecting the vaginal smear in the castrated rat. This includes the lipoid extracts with which McGee was able to produce the growth of the male secondary characters in capons and which, therefore, supposedly contain assayable amounts of male sex hormone.

Loewe (309) who likewise assayed male sex hormone (Androkinin) by means of the Loewe and Voss "Zytologisches Regenerationtestis" was unable to produce an effect on female castrated mice.

Biedl, 1927 (43) from the results of parabiosis between male and female rats, in which the cycle stops until the union is severed, concludes that the male hormone counteracts and neutralizes the female sex hormone. His conclusions are weakened by his own statement that thyroid extract, for example, stops estrus in normal rats.

My criticism, made in connection with the test for so-called inhibitory substances contained in the corpus luteum, applies as well here. The cycle is so readily affected by non-specific interventions that its regularity is an unreliable test.

Hormone Sterilization, so-called as described by Haberlandt (197–8) and others, appears to me to be due, as R. Meyer also believes, 1928 (338), to parenteral foreign protein effect.

Antimasculin Effects of the Female Sex Hormone

Even more discordant are the reports obtained upon the "antimasculin" effect of the female sex hormone. Some of this confusion may be due to a difference in effect produced on the immature and mature male genital tract. It must also be remembered that the testis is sensitive to such influences as comparatively slight changes in temperature and to foreign protein.

In 1916 Herrmann and Stein (219) found that the lipoid extract of corpus luteum not only exerted an inhibiting effect on the growth (size) of the immature testis of rabbits but also on spermatogenesis. The resulting testis was like an X-rayed testicle. In older animals, if spermatogenesis had begun, regression took place (220, 428).

Truffi, 1927 (444) and Laqueur, 1926 (263, 268) have observed a distinct antimasculine effect exerted by the female sex hormone, the latter stating that as the result of three weeks of treatment the testes, penis, prostate, and seminal vesicles regress, the testis canals proving empty. Laqueur, in particular, emphasizes that injection of female sex hormone into immature males, produces a great lagging behind of the accessory male appendages. If the injections are discontinued, a rapid return to normal may result.

Reiprich, 1925 (385) has called attention to the physiological involution of the fetal testis during the last two months of intra-uterine life. According to him, this regression persists for some time after birth, in despite of the withdrawal of female sex hormone of the mother. To this must be added the claim of Fels (121) that the serum of pregnant women causes retardation of growth in the immature (but not adult) testis. Lipschütz, too, 1926 (293) has obtained antimasculin effects.

Yet such apparently conclusive evidence must be accepted, or at least scrutinized, with caution and reserve when Heredia (216) found that male chickens fed with bull testes had testes smaller than the controls, though their secondary sex characters remained male. Kauders (248) fed testicle extracts in "overdosage" and obtained atrophy of the rat testis. These results speak for sensitiveness of the male gonad to many influences rather than to any specific contrasexual effect.

Steinach *et alia*, 1928 (430) now state that cattle placenta up to 500 M. U. pro die has no antimasculin effect.

An antimasculin effect is particularly disconcerting in view of the fact that Dohrn (95) and Hirsch (222) as well as Frank and Goldberger (160) have demonstrated recognizable amounts of female sex hormone in the blood of potent males, and that Laqueur (267) as well as others, have been able to isolate titerable amounts of female sex hormone from the urine of males. Our own (Frank and Goldberger, unpublished) repeated investigations performed on 15 male urines have, however, proved negative.

It seems strange that, if the female sex hormone produces an antimasculin effect, it should be found as a normal, even if only occasional, constituent of the male blood and urine. It is of course possible that the small quantities transiently present produce no deleterious effect on the function of the testes. This is particularly possible in view of the success with which Lipschütz (293) has been able to implant ovarian grafts in male guinea pigs whose testes continued to function, as proved by the fact that they succeeded in impregnating females, and in which the breast and nipple enlargement with milk secretion documented the functional persistence of the ovarian graft.

Effect on the Ovaries

All the earlier investigators, including Aschner (27), Schickele (400), Okinschitz (351), Frank and Rosenbloom (165) with the exception of Herrmann and Stein (219–20), who described a stimulation of follicle growth in the ovaries of their adult rabbits, agreed that injection of the active substance had no effect on the ovaries of either immature or mature animals. The more recent investigations have sustained this contention with the exception of Laqueur (271) who mentions that the ovaries contain *less* follicles after female sex hormone has been exhibited for any length of time.

Figure 28, page 68, shows the ovaries of an immature rabbit treated with female sex hormone for seven days. Contrasted with that of an untreated immature control no difference is noted.

This lack of influence on the female gonad itself is in marked contrast to the rapid and striking activation of follicle ripening and luteinization which results from injection of anterior pituitary lobe extracts or implants in both premature and adults, (see Evans and Burr (109), Zondek and Aschheim (486), Philip Smith (424).

Murata and Adachi (344) noted that injection of ether extracts obtained from early human chorion, produced effects such as the above. This confirms the claims of Aschheim (22) that the placenta contains anterior lobe hormone. The Berlin author believes that the lutein cystic changes in the ovaries, noted in chorion epithelioma and hydatid mole, are due to overproduction of the anterior lobe hormone.

Discovery of the Female Sex Hormone in the Blood

In 1905 Marshall and Jolly (325) injected the blood of estrous bitches into other, anestrous bitches, and claimed to have obtained immediate flushing of the vulva in the anestrous animal.

In 1913 Schickele (400) attempted to produce uterine growth phenomena in rabbits by injecting the blood serum of pregnant rabbits, with negative results.

Fellner, 1913 (114) using 5–10 c.c. of pregnant rabbit's blood, injected a 2500 gram rabbit, obtaining doubtful results on the uterus. He, therefore, concluded "that larger amounts of active substance are not contained in the blood of the pregnant rabbit."

F. Binz in 1924 (46) published a small and apparently unnoticed communication in the Münchener Medizinische Wochenschrift dealing with the production of signs of premature puberty in infantile mice following injection of the blood serum of pregnant women. He used 0.5 c.c. injected daily for 8 days. It is surprising that this important observation passed without notice or comment for several years. The work was called to my attention by a personal communication from Dr. Binz more than a year after our first reports appeared in print.

Loewe of Dorpat on July 16th, 1925 (305) announced the recovery of active substance obtained from the blood of a female rabbit, cow, and a woman. The activity was demonstrated by the Allen and Doisy test. Less than four weeks later appeared the publication of my group, Frank, Frank, Gustavson, and Weyerts (154) in which we announced the recovery of the active substance from the blood of estrous sows, its absence in the non-estrous animal, and in bulls' blood. We have pursued this work in many directions. The technic will be described in detail in the second half of this monograph which deals mainly with the clinical investigation based on the test elaborated by us.

Since 1925, then, Frank and Goldberger (155–160) have studied the variation of the female sex hormone in the circulating blood of women at different stages of the cycle, establishing norms. We have also described the continuation of a high hormone level in the circulation from the 7th week of pregnancy on to term (157–8). Further we have shown that the menstrual blood contains a concentrated amount of female sex hormone (155). By means of this reaction, after normal conditions had been sufficiently studied, we investigated various pathological complexes in the human female (158). This work has been conducted on a large scale. As yet few others have published on this method although partially confirmed by the report of the female sex hormone in the blood of pregnant women by Trivino, 1926 (443) who used Binz' technic, and with 43 sera of pregnant women, induced premature maturity in 42 cases in immature mice. Fels (120) also, from the clinic of Fraenkel as well as the report of Margaret Smith (419) from the Obstetric Service of Johns Hopkins Hospital, fully confirm our results on pregnant women. Hirsch, 1928 (223) has used our method in 68 examinations of the blood, with results identical with our own. Numerous workers both here and in other countries, have come to us to learn the method and are pursuing investigations with this method, but have not as yet published their results.

The Female Sex Hormone in the Urine

Loewe (311) in 1926, discovered the female sex hormone in the urine. He found that during the cycle, at most one to two mouse units per litre could be obtained, at about the 13th day after the onset of the previous menstruation (10th day of the new cycle). Laqueur (264) on the other hand reports finding as much as 200 M.U. pro liter on the second day of the menses. He does not state whether the urine was obtained by catheter, uncontaminated by menstrual blood. We find an intermediate rise, which is in agreement with my view that at the time of impending menstruation the kidney and intestinal threshold for female sex hormone is abruptly lowered (see page 191).

Zondek (482) on the other hand discovered huge amounts of hormone in the urine of pregnant women. This excess of excretion was found from about the eighth week on, extending to term. Throughout this time, from six to seven thousand mouse units, with a maximum of ten thousand mouse units per liter, were noted. He therefore injected from 1–3 c.c. of undiluted urine into a castrate mouse, a positive reaction indicating the existence of pregnancy.

Our own experiments have indicated a lower concentration in the urine of pregnant women. We have rarely found as little as 5 c.c. of pregnancy urine to contain 1 M.U. If such urine is extracted with ether at least 15 c.c. of urine must be used to obtain a positive reaction with the ethereal extract. For details see Part II, page 196.

Mazer and Hoffman, 1929 (330), attempt to diagnose pregnancy in the early weeks by injecting 20 minims of freshly catheterized urine every 2 hours for 5 times, into castrated mice. A positive vaginal spread is regarded as evidence of pregnancy. In 67 tests on pregnant women 61 were positive, the negative being in the first five weeks of gestation. Of 142 control tests, 15 were positive. They concede an error of 8 per cent. Even with this their test may prove of value.

It is thus apparent that an enormous overproduction of female sex hormone takes place throughout pregnancy. For this reason Zondek and Aschheim (482) have proposed the urine of pregnant women as a convenient *source* for obtaining the hormone (for technic see page 141).

Female Sex Hormone in the Feces

Dohrn and Faure (96) report that large amounts of hormone are excreted in the feces. The excretion may reach 30,000 M.U. per kilo of dry feces.

Female Sex Hormone in Males

The report by Dohrn (95) as well as Hirsch (222), that extracts obtained from male blood may produce a positive estrous reaction in the castrated mouse, surprised us greatly. The hormone, or a body acting like this hormone, has been isolated from the male blood as well as from the male urine, Loewe, Voss, Lange, and Wähner (318). Our (160–1) own investigations have convinced us of the correctness of their observation. This occasional finding does occur. In 47 men we obtained three threshold reactions and 4 weak reactions.

Laqueur (267) has obtained as much as 50 M.U. of female sex hormone per liter of urine from men. I am not ready as yet to explain this anomalous and disconcerting discovery, particularly as very careful study of testicular extracts has proved negative in our hands in spite of the positive report of Fellner (115) and Carminati (70) who described such reactions obtained with testicular extracts. Carminati's work we repeated most scrupulously, always with negative results. We then injected five times the amount of rat testis extracts into castrate female rats and protracted the period of injections far beyond what he had practised. The results always were negative.

The explanation naturally suggesting itself is in favor of the theory of a quantitative and fluid transition from male to female, (see page 8) and that such individuals possess hidden female biological qualities, thus again offering, with due reserve, a possible explanation for homosexuality. In view of the constantly reiterated antimasculin effect of female sex hormone by Laqueur (274), Herrmann and Stein (219) and others, it is hard to conceive that both hormones could be present in the same individual without mutually interfering with or neutralizing each others actions. However, Steinach (429) and Lipschütz (293) have succeeded in having the active gonads of both sexes developing and exerting at least some of their biological effects in the same animal (spermatogenesis, activation of breasts). Halban (201) on the other hand, believes that the male and female sex hormones are almost identical in structure and properties. For the moment the question must be left open and unanswered. We are actively engaged in the study of this puzzling contradiction.

Presence of the Female Sex Hormone in other Tissues

In the Bile

Gsell-Busse, 1928 (194) has obtained a positive reaction from bile, whether this was obtained from human or animal sources, from males or from females. A positive reaction was obtained with the bile of senile men in the eighth decade of life. We have been able to verify this statement in our laboratory and have likewise confirmed his finding that the ether extract of sodium taurocholate gives a similar reaction. Perhaps the positive results in the experiments of Schröder and Goerbig (410) in which liver extracts were used, gauged by the growth of rabbits' uteri, are accounted for by the presence of bile extracts contaminating the liver extraction. As yet, repeated use of liver extracts have never given me a positive reaction, whether I employed rabbits' uteri or the Allen and Doisy test as a determinant.

In Plants

The female sex hormone has been demonstrated in plants from the yeast organism up. Fellner, in 1921 (115) announced its presence in yeast, using the rabbit's uterus for the test. In our laboratory (unpublished) one M.U. was obtained from 350 gm. of wet yeast, extracted with alcohol and ether after drying with anhydrous sodium sulphate. Our efforts to show its presence in bacteria have as yet been of no avail, perhaps because of the insufficient amounts of culture material available.

> Through the kindness of Dr. Schwarzman, bacteriologist of Mount Sinai Hospital, large quantities of bouillion cultures of bacillus pyocyaneus and bacillus coli were obtained and extracted with alcohol and ether. These extracts proved negative.

Loewe (306, 316) has demonstrated a strong female sex hormone reaction in extracts from the buds of female willows. He found that more female sex hormone was obtained from the buds than from the stems and other portions of this tree, as well as from the yellow pond lily (*nuphar luteum*).

Faure, Dohrn, Poll, and Blotevogel (97) have shown the presence of the hormone in potatoes, sugar beets, and sugar-beet seeds. Fellner (117) has shown its presence in rice and wheat.

Lecethin from hen's eggs assayed by means of the rabbit's uterus reaction, according to Jaffé, 1926 (244) showed this same growth substance. Allen *et alia* (12) found it in the follicle fluid of the hen. We, Frank, Gustavson, and Goldberger (unpublished) have found activity in the yolk of hen's eggs.

It is of importance to recognize the fact that, although the various reactions elicited by the hormone obtained from plants are *almost* identical with that of hormone obtained from animals, female sex hormone from plants produces an estrous reaction in mice which is slower in developing, more persistent, and occasionally of more intermittent character than when due to the active substance obtained from animal sources.

This feminine principle which we call the female sex hormone is widely distributed throughout the vegetable and animal kingdoms. It would appear that at first, as in the yeast, the active substance is a mere growth-stimulating principle; that gradually as we trace it through the higher plants it becomes more and more localized in the specifically generative portions of the plant. Finally in the animal kingdom the hormone is increasingly centralized in the ovary and its action is more and more specifically limited to the generative tract, including the mammary glands. This corresponds closely to the generalization enunciated by Kohn of Prague (254) who considers all endocrine secretions in the nature of general metabolic products which later become more and more specialized in their action and limited in their sphere. This generalization of mine may be, as future research will either prove or disprove, somewhat premature and speculative. It may have to be modified in many of its details. Nevertheless it appears to explain the philogenesis of the female sex hormone.

I cannot agree with investigators like Loewe (317) or Laqueur (264), for example, who attempt to explain growth of the uterus as due to a part component of the female sex hormone, a "growth-stimulating oil" ("Wachstumsoel") and a second, an estrous-inducing component assayable by the vaginal smear reaction. All the evidence goes to prove that the *same active principle*, as the result of quantitative differences in dosage, produces diverse phenomena.

Absence of Species Specificity

It has been demonstrated that the female sex hormone has no species specificity because follicle fluid, placenta, and corpus luteum have furnished extracts when derived from the most diverse origin (varying from hen to the human) which act upon such diverse species as dove, rat, and monkey. Even the extracts obtained from the plant kingdom act upon

TABLE I

Distribution of the Female Sex Hormone

M.U.Kg. = Mouse unit per kilogram. R.U.Kg. = Rat unit per kilogram.
M.U.L. = Mouse unit per litre. R.U.L. = Rat unit per litre.

SOURCE	TYPE OF TEST	AMOUNT	YEAR	AUTHOR
Yeast	Rabbit's uterus	+	1921	Fellner
Yeast	Vaginal smear	7 M.U.Kg.	1928	Frank and Goldberger
Buds of salix caprea	Vaginal smear	48–200 M.U.Kg.	1926	Loewe, Lange, and Spohr
Althea rosea	Vaginal smear	3 M.U.Kg.	1926	Loewe, Lange, and Spohr
Stems of patiens parviflora	Vaginal smear	1 M.U.Kg.	1926	Loewe, Lange, and Spohr
Ovary: nuphar luteum	Vaginal smear	12–20 M.U.Kg.	1926	Loewe, Lange, and Spohr
Sugar beet	Chrom reaction	+	1926	Dohrn and Faure
Potato	Chrom reaction	+	1926	Dohrn and Faure
Parsley	Chrom reaction	+	1926	Dohrn and Faure
Cherry	Chrom reaction	+	1926	Dohrn and Faure
Plum	Chrom reaction	+	1926	Dohrn and Faure
Wheat, rice	Rabbit's uterus	+	1925	Fellner
Fish roe	Rabbit's uterus	+	1925	Fellner
Hen's eggs	Rabbit's uterus	+	1925	Fellner
Yolk of hen's eggs	Vaginal smear	+	1927	Frank, Goldberger, and Gustavson
Ovary of sow	Rabbit's uterus	+	1912	Iscovesco
Ovary of cow	Rabbit's uterus	+	1913	Herrmann
Ovary of bitch	Rabbit's uterus	+	1916	Frank
Ovary of human	Vaginal smear	+	1925	Allen and Doisy
Corpora lutea	Rabbit's uterus	+	1912	Iscovesco
Corpora lutea sow	Rabbit's uterus	+	1913	Herrmann
Corpora lutea cow	Rabbit's uterus	+	1915	Frank and Rosenbloom
Corpora lutea hog	Vaginal smear	170 R.U.Kg.	1925	Frank and Gustavson
Corpora lutea human	Vaginal smear	2–3000 R.U.Kg.	1925	Allen, Pratt, and Doisy
Follicle fluid cow	Rabbit's uterus	+	1922	Frank
Follicle fluid sow	Vaginal smear	2100 R.U.Kg.	1923	Allen and Doisy
Follicle fluid mare	Rabbit's uterus	+	1923	Seaborn and Champy
Follicle fluid hen	Vaginal smear	+	1924	Allen

TABLE I (Continued)

SOURCE	TYPE OF TEST	AMOUNT	YEAR	AUTHOR
Follicle fluid human	Vaginal smear	7390 R.U.Kg.	1925	Allen, Pratt, and Doisy
Ovarian residue	Vaginal smear	60 R.U.Kg.	1924	Allen and Doisy
Ovarian cyst fluid		+	1923	Papanicolaou and Blau
Ovarian cyst fluid	Vaginal smear	250 R.U.Kg.	1926	Frank and Goldberger
Placenta term	Vaginal smear	300 M.U.Kg.	1926	Loewe
Placenta 4th month	Vaginal smear	150–200 M.U.Kg.	1926	Loewe
Blood, human	Vaginal smear	+	1925	Loewe; Frank
Amniotic fluid	Vaginal smear	4 M.U.Kg.	1926	Brouha and Simonnet
Cord blood	Vaginal smear	+	1926	Loewe
Urine ♀	Vaginal smear	2–7000 M.U.L.	1926	Loewe; Aschheim,
Urine ♂	Vaginal smear	50 M.U.L.	1927	Laqueur
Feces	Vaginal smear	30,000 M.U.Kg.	1928	Dohrn and Faure
Bile ♀ and ♂	Vaginal smear	600 R.U.Kg.	1928	Gsell-Busse
Testis	Rabbit's uterus	+	1925	Fellner
Testis	Vaginal smear	+	1927	Laqueur

the animal organism. Tables I and II show these facts in a short and graphic fashion.

Sex Specificity

Certain tissues react in a fashion which appears to warrant the assumption of an asexual soma. A most striking example of response to a hormone by well-differentiated glandular structures is furnished by the breasts of males and females, and by analogous organs of the two sexes, the penis and clitoris.

The breast tissues of both males and females respond equally well to the growth impulses initiated by the female sex hormone. After the growth has reached a certain degree, withdrawal of the stimulus is followed by secretion of colostrum or milk in either sex.

The phallus of both sexes responds to male stimuli. The clitoris in in particular, although it has not been directly influenced by any obtainable male sex hormone, shows increased growth and development simultaneously with the exaggeration of other male secondary characters, as, for instance, the hair development, under the stimulus of adrenal tumors (245).

TABLE II

Material Investigated by Frank and Goldberger

MATERIAL	M.U.KG.	R.U.KG.	
Yeast	7		
Fish Roe		40	
Goat's milk (estrus)	1000	400	with Gustavson
Pig's fetuses ♂	+		
Pig's fetuses ♀	+		
Pig's fetuses liver ♂	+++		
Pig's fetuses liver ♀	+++		
Placenta term human	1000	540+	
Placenta 1st month human		100	
Placenta, cat		40	
Placenta, beaver		80	
Placenta, guinea pig		250	
Corpus luteum human	500		
Corpus luteum sow, yellow		170	Frank and Gustavson
Corpus luteum sow, yellow pink		150	
Corpus luteum sow, pink		110	
Corpus luteum sow, bloody		60	
Follicle fluid		1000	
Simple ovarian cyst		250	
Corpus luteum cyst	350		
Human amniotic fluid	300		
Human decidua 4th month		100	
Human blood, non-pregnant	10–50		
Human blood, pregnant	50–100		
Human urine, non-pregnant	0–5		
Human urine, pregnant	300–500		
Human bile ♂ and ♀		100	

The sex specificity of the female sex hormone has been particularly studied of late and has been called into question for reasons which I shall elaborate.

As mentioned on page 11 the female sex hormone of the mother activates the breasts of both the male and female fetus *in utero*. It would appear from our investigation (unpublished) of fetal extracts that much of the female sex hormone circulating through the cord blood is taken up and stored in the liver. As was mentioned previously, the uterus, the prostate, and the breasts of fetuses near term and of the newborn, have reached a much greater stage of development than those of the same infant some 2 to 3 months after birth, when the activation produced through the mother's circulation has disappeared.

At puberty, as was mentioned on page 14, through the intermediary of the anterior lobe activation, the ovaries become functional. As yet, the sole explanation at all acceptable has been offered by Evans and Simpson (110) who believe that the sex growth-producing anterior lobe constituent is not available until the demand of the general soma for growth-producing factors has ceased or become less powerful.

At puberty the effect of this sudden production, which releases larger quantities of the female sex hormone, is perceptible in the sudden increase of uterus, vagina, and breasts with the appearance of the first estrus, or in the human, of the first menstruation. This is followed in due course, if physiological forces are permitted a free reign, by copulation and pregnancy.

All evidence favors the conception that even before the first appearance of estrus, perhaps due to a smaller amount of anterior lobe activation, a non-cyclical increase of ovarian production of female sex hormone takes place, in sufficient amount to stimulate the secondary female sex characters, especially those which bring the bone growth to its termination, which influence the appearance of the pubic and axillary hair, and prepare for the cyclical changes.

In certain species, special sexual sex characters of a strikingly reversible kind have been described (see Tandler and Grosz, 437). Such are the horns of cattle in the deer family, and the feathers of birds, which appear to be bipotential and directly influenced by the amount and character of the sex hormone (Lillie, 285). This brings us directly back to the previously mentioned experiments dealing with sex reversal (see page 7).

Possible Explanation of the Occurrence of the Female Sex Hormone in Males

The sole apparent exception to the sex specificity of the female sex hormone has been due to the discovery of a principle in the blood, urine, and bile of males which gives an identical reaction with that found in females (see page 114). Thus the anomalous condition exists of finding a female sex hormone, which, according to much evidence, is antimasculin in its action, in the bodies of males whose masculine characteristics and ability to impregnate females is unquestioned. The answer to this paradox appears simple to me. Occasionally, but only occasionally, in the same male, sufficient female sex hormone can be isolated from the blood (Frank and Goldberger, 160) or from the male urine (Laqueur, 267). But from the male bile (Gsell-Busse, 194), including even the bile of senile men in the 8th and 9th decade of life, female sex hormone can always be extracted.

It should be noted that female sex hormone or female sex hormone-like substances occur in considerable concentration in all common food products like yeast, the potato, sugar beets, caviar, and other fish ova, and probably also in the liver (Schröder and Goerbig, 410). If there is no utilization of this substance in the male, two possibilities arise, the one that the substance is either not absorbed from the intestinal canal, or, if absorbed, is at once excreted through the urine and feces; the second, that it passes the intestinal wall but is then kept from entering the general circulation by some protective mechanism.

This last possibility is strengthened by the fact that large doses of female sex hormone given *per orem*, produce little effect unless excessive amounts, such as 100 times the parenteral dose, are received. We note from the studies of Giesy (182) with the soy bean, from Doisy and Allen with pancreas hash (93), from Laqueur (263) who employed pancreas enzyme and also pepsin, that the hormone is at least strongly resistant to certain enzymatic influences. This, together with the fact of its concentrated appearance in the male bile, has led me, upon purely theoretical grounds, to conclude that the female sex hormone is readily absorbed from the intestinal tract, but reaches the liver and is there excreted into the bile in which it is stored. Therefore, only on certain occasions which may be either the sudden emptying of the gall bladder for example, in which case the female sex hormone directly passes to the duodenum and is excreted by the feces, or when a too high concentration is reached in the biliary fluids, the female sex hormone is expelled by secretion into the blood and then excreted through the kidneys by this optional route. We (Frank and Goldberger) are at present engaged in putting this working hypothesis to the test of experiment.

TABLE III

Negatives by Rat Vaginal Smear Method, (Frank, Goldberger & Gustavson

Bacillus coli
Bacillus pyocyaneus
Tomato juice
Bios I
Bios II
Radiated cholesterol
Bull's testis
Rat's testis
Histamine
Adrenalin
Aolan
Thymus gland
Adrenal gland

Anterior lobe pituitary
  acetone extract
  aqueous extract
  saline extract
  from pregnancy urine
Thyroid gland
Liver, cattle
Brain, cattle
Pseudomucin cyst fluid
Dermoid cyst fluid
Serous cyst-adenoma fluid
Human sweat (small quantity)
Human (pregnancy) saliva (50 c.c)

Results with Other Methods of Investigation

In Vitro Growth. Frank, in 1911, together with Lambert (135) succeeded in growing rat endometrium *in vitro*. As a rule, connective tissue growth only was obtained, but occasionally epithelium could be cultivated. It was found that addition of small amounts of corpus luteum to the culture did not stimulate either connective tissue or epithelial growth.

Courrier (78) in 1925, grew the vaginal epithelium of castrate female guinea pigs *in vitro*, but likewise was unable to stimulate this epithelial growth by the addition of female sex hormone in the form of follicle fluid.

Intra-ocular Growth. In 1920 Schochet (405) published experiments with material grown in the anterior chamber of the eye. Markee, 1929 (323) has studied the uterine endometrium of the guinea pig under these conditions. He finds rhythmic blanching and flushing. Even greater differences result from the estrous changes. During full estrus the tissue remains constantly flushed, in contrast to the rapid alternation otherwise observed. It is the small capillaries which dilate and contract, approximately every 15 seconds. The author suggests this as a possible test for female sex hormone.

The Implantation Method. Zondek and Aschheim (483, 487), as described previously on page 48, implanted minute quantities of the tissues to be investigated into the thigh muscles of castrated mice. The hormone content of the implant enters the circulation of the host by osmotic processes, thus if positive, affording a delicate and accurate method of qualitative assay of a given tissue. These authors controlled the type of tissue implanted by microscopic examination of adjacent fragments. By this modification of an old device they were able to determine that the granulosa layer, the lutein layer of the corpus luteum, the theca interna as well as the diffuse cortical tissue (in pregnancy only) in the human ovary contained female sex hormone. They were able also to show that minute quantities of anterior lobe pituitary, whether derived from male or female sources, human or animal, were able to induce premature puberty in the immature, as well as luteinization in the adult ovary. They claim, however, that in the castrate, no effect follows such implantation. With this view P. Smith (423) is also in accord.

This last is contrary to the reports of Brouha and Simonnet (61) who have described the induction of estrus in consequence of injection of acetone extracts of the anterior lobe pituitary in castrate rats.

Our own (Frank and Goldberger, unpublished) attempts to verify this effect on the castrate have repeatedly failed, although the extracts were prepared according to the verbal directions of Brouha himself. (See page 15).

Induction of Menstruation in Castrates

Zondek, 1926 (481) by means of his folliculin B, an impure water soluble preparation of female sex hormone, was able to induce menstruation twice in a woman castrated two years previously.

More readily analyzable results were obtained by Allen and Pratt (5, 378) who performed 13 series of injections in 5 spayed monkeys. Each of these animals received between 64 and 195 R.U. of female sex hormone in the course of 9 to 20 days. In 7 of 10 series, bleeding followed the discontinuation of the active extract, the bleeding lasting from 1 to 6 days.

These results definitely show that for the induction of menstruation, the activation of the uterus by means of the female sex hormone is alone necessary, the catabolic processes following automatically upon the sudden withdrawal of the active substance from the sufficiently "loaded" endometrium.

Comparison of Female Sex Hormone to Vitamins

Laqueur (271) emphasizes that the female sex hormone produces its effects upon Müller's tract in smaller dosage than is needed to produce specific effects with vitamin B and D.

> For example, of vitamin D (radiated ergosterol) 0.001 *pro die* or 0.01 mg. in 2 weeks is required to cure rachitis. Similarly, in small birds of identical weight as rats, a daily dose of 0.001 mg. vitamin B is needed to prevent beri-beri. With as little as 0.00007 mg. menformon Laqueur obtained uterine growth effects in rats.

A year before this publication, we (Frank and Goldberger) had put this question to the test of experiment, see Hess, Weinstock, and Sherman, 1926 (221). Large quantities of activated cholesterol (supplied by Hess) gave no female sex hormone reaction nor did female sex hormone (supplied by Frank) produce any antirachitic effect.

Neuweiler, 1928 (346) has employed vitamins B-C-E on castrates without inducing estrus.

According to R. Meyer (338), Hirsch-Hoffman, with "Vigantol" (Merck) which is equivalent to vitamin D in oil, was able to produce hypertrophy of the uterus in castrated rats. I know nothing concerning the origin or derivation of this product.

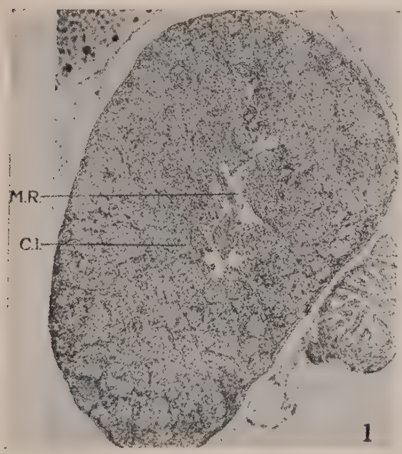

Fig. 42. A. Ovary of 3 Week Old Mouse Exposed to Full Sterility Dose of X-ray. ×50.

Shows maximum development of first proliferation arranged in thick finger-shaped cords. M. R.—Loose medullary region. C. l.—Cord of first proliferation.

(From Brambell, F. W. R., Parkes, A. S. and Fielding, U. Proc. Roy. Soc. B., 1927, 101: 29.)

Parkes (364) in 1928 mentioned that vitamin B deficiency in mice is followed by anestrus. However, such animals respond as readily to the injection of female sex hormone by showing estrus, as a castrate would, this signifying merely that the ovaries are inactive in these deficient animals.

Frank and Goldberger (unpublished) have found Bios (both "Crude" and Bios II) inactive. In all we may now state that at least vitamins B, C, and E in enormous dosage produce no female sex hormone reaction in the castrate rat or mouse.

X-ray Effects

Holzknecht (228), Geller (179), and others, have studied the effects that X-ray produced on the ovary. One and all have failed, by means of this method, to obtain destruction of individual elements in a sufficiently striking fashion to permit of analysis of ovarian function. Parkes and Bellerby on the other hand, in a prolonged, elaborate, and apparently

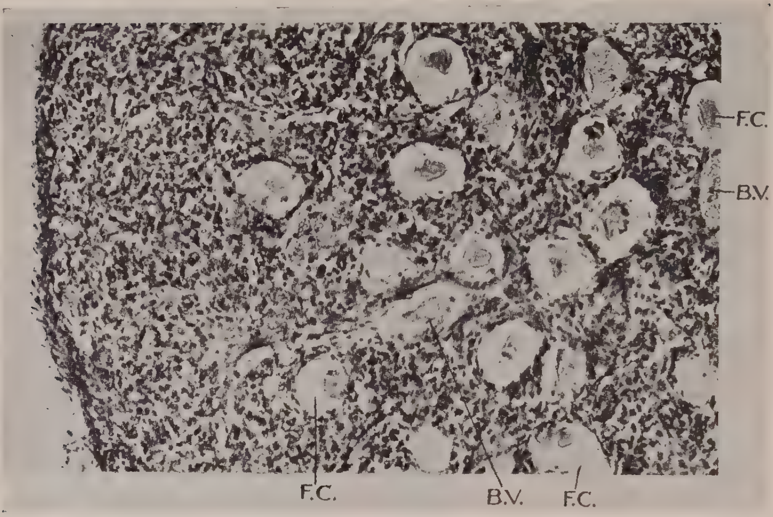

FIG. 43. B. M 2c SHOWING CAVITIES CONTAINING ZONA PELLUCIDA REMNANTS AND NECROTIC FOLLICLE CELLS BEING PUSHED AWAY FROM PERIPHERY BY GROWTH OF THE FIRST PROLIFERATION. ×200.

F. C.—Cavity representing old follicle. B. V.—Blood vessel.

(From Brambell, F. W. R., Parkes, A. S. and Fielding, U. Proc. Roy. Soc. B., 1927, 101: 29.)

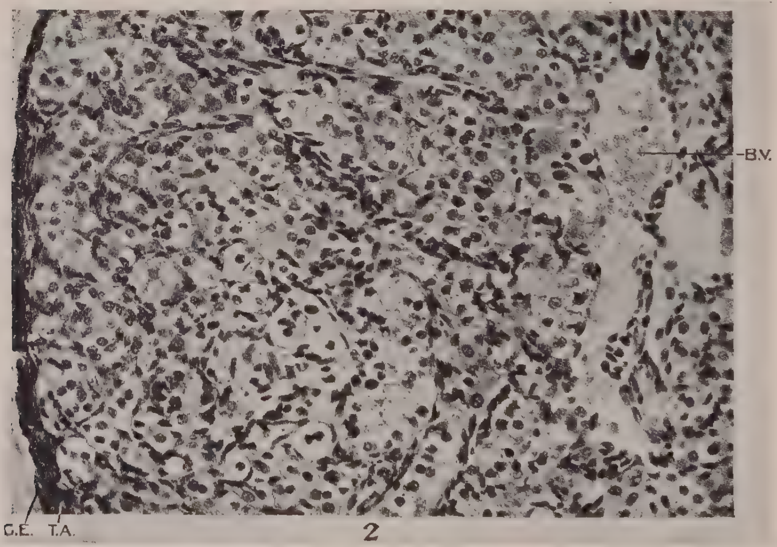

FIG. 44. C. M 7A SHOWING ENLARGED VACUOLATED TISSUE OF THE FIRST PROLIFERATION, THIN GERMINAL EPITHELIUM AND WELL-DEVELOPED TUNICA ALBUGINEA. ×290.

T. A.—Tunica albuginea. B. V.—Blood vessel. G. E.—Germinal epithelium.

(From Brambell, F. W. R., Parkes, A. S. and Fielding, U. Proc. Roy. Soc. B., 1927, 101: 29.)

well controlled series of experiments, dating from 1926 to the present day, claim by means of radiation, to have destroyed all ovogenic tissue in the mouse's ovary (359–363). They claim therefore to have abolished alternating growth of follicle and corpus luteum and yet, according to their observations, such "sterile" mice, by means of the vaginal spread reaction, in many instances show a cyclical variation. Examination of the ovaries of these animals (55) shows a downgrowth of germinal cords from the surface of the ovary. Mice, which have been irradiated at or before birth (360, 361), eventually develop the signs of puberty and cycle.

As a result of their experiments, Parkes and Bellerby conclude that the alternating growth of follicles and corpus luteum is not necessary for the production of the cycle. On the contrary they believe that the cyclical action results from the periodic attainment of a threshold value of the female sex hormone. The persistence of the corpus luteum, to which they ascribe an inhibiting action, has the effect of delaying the attainment of this threshold, and of throwing the cycle out of gear as during pregnancy, pseudo-pregnancy and lactation. As "at the same time oestrin can be readily extracted from the ovaries of pregnancy animals, it is necessary to suppose that the oestrus inhibitory action of the corpus luteum exerts a dominating action during pregnancy. The exact nature of this inhibitory action is not yet clear but it is plain that it is not localized to the ovary itself."

I must confess my inability to follow the reasoning of Parkes and Brambell, based on their results with X-rayed ovaries. The one outstanding fact is that the cycle continues. It is true that scrutiny of their reports (55) shows that the cycles appearing after "sterilization," by raying of the ovaries, are far from regular. For example, of 25 mice, 4 only had 3 cycles, 5 only had 2 cycles, and 9 only had 1 cycle while the length of the cycle varied anywhere from five and one-half to 33 days although in a later paper the study of 1000 normal cycles, Parkes, 1928 (365) showed a *mean* of 6.2 days.

Allen (3) demonstrated that cyclical ovogenesis takes place in the adult mouse. Papanicolaou (353) in 1924 noted much greater post-puberal ovogenesis in guinea pigs whose thyroid had been treated by radium than in the normal in which he only occasionally found ovogenesis. It therefore seems fully justified to suspect that what is taking place in the rayed mice studied by Parkes (and Brambell) is a preparation for the growth of follicles and a consequent production of female sex hormone by this germinal tissue.

Parkes and Brambell have succeeded in destroying the follicles and sometimes also the corpora lutea in the ovary. This effect is followed by regenerative processes which in turn produce female sex hormone. From their results they are at least justified in postulating an emergency substituting apparatus for the normally present follicles and corpora lutea. That their assumption of a threshold mechanism is likewise warranted is fully in accord with my own views, based on entirely different data, to which I shall refer in connection with the amount of female sex hormone present in the blood and urine of the cyclical human female (see page 191).

On the other hand, when Brambell and Parkes (54), noting the previously well known fact, that even after double-oöphorectomy (if performed on a mouse not longer than two days before estrus is due) the last estrus is not prevented, conclude that "follicular maturation is not responsible for oestrous production and suggest that follicular maturation itself results from the operation of the oestrous-producing stimulus," they appear to confound cause and effect. Injection of follicle fluid ordinarily requires 48 hours to act in the mouse. The female sex hormone has already sensitized the mouse's uterus when the ovaries are removed. That is the only deduction warranted.

These supposedly "sterile" animals are, however, I suggest, only temporarily sterilized. If Parkes and Bellerby will follow these animals further, they may note full regeneration of the follicle apparatus from the downgrowing epithelium and a greater or lesser *restitutio ad integrum*. On page 252 I refer to blood investigations conducted on women whose ovaries were subjected to X-ray, in the pregnant and non-pregnant state. In both, an early disappearance of a threshold amount of female sex hormone is noted in the blood of those who received sufficient radiation, and a reappearance of the hormone can be observed when the ovarian activity returns.

The Number of Hormones Elaborated by the Ovaries

Only the briefest summary of this moot question can be attempted here.

1898–1912. The early theorists and investigators such as Prenant (379), Beard (37), Fraenkel (127), and Loeb (298) believed that the corpus luteum was *the* ovarian hormone.

Later Halban (200) suspected and Iscovesco (231) and others showed that a hormonal effect could be obtained from the ovary and placenta. Loeb especially emphasized the inhibiting and sensitizing importance of corpus luteum while Halban (200), Adler (2), Frank and Rosenbloom (165) and others were interested in studying the anabolic growth effects of extracts.

Since 1923 the spotlight has been turned mainly upon the follicle extracts, often to the exclusion of the other sources of the female sex hormone.

Marshall (326) believes that there are 3 ovarian hormones, the follicular, governing estrus; the corpus luteum, regulating the periodicity and the early nidation of the ovum; and a third hormone produced by the thecal cells which governs the trophic growth of the genital tract.

I feel that the theoretical deductions of Marshall are not justified by the facts, which may be explained on the basis of quantitative and time reactions instead of by a third hormone.

Papanicolaou (354) agrees vaguely with Marshall, in that he claims to have obtained a lipoid fraction from the corpus luteum, which causes regression of the follicles and suppression of ovulation, a water soluble fraction which increases ovarian functional activity, and finally a follicular fraction which causes the estrous reaction.

Laqueur (263) also, is uncertain as to the number of hormones elaborated. The corpus luteum contains little. Menformon by mouth has little effect and yet exhibition of dried extracts helps castration and menopause discomforts. From this inconclusive evidence he deduces the possibility of several hormones.

My own convictions lead me to believe that the female sex hormone is the anabolic hormone which produces all the growth phenomena in the Müllerian tissue and the breast. The type of phenomena resulting depends upon the amount acting upon these tissues, as well as the time during which the action is maintained.

Until a few weeks ago data permitting a final decision upon the presence of a second, special inhibiting and sensitizing corpus luteum hormone were lacking. Our (Frank and Goldberger, unpublished) numerous experiments with corpus luteum extracts, prepared according to the method of Parkes and Bellerby (370) and Hisaw (225a) have uniformly proved negative. However, in my opinion, the original experiments of Loeb (298) from 1908 on to 1928 show conclusively that the corpus luteum does inhibit ovulation and sensitizes the endometrium. Even if extracts have as yet failed to reproduce these reactions in a convincing fashion, too many facts attest to this special corpus luteum function to permit me to doubt of it.

The recent experiments of Corner and Allen, and Allen and Corner, have supplied much additional proof. In addition we (Frank, Gustavson, and Goldberger) have now obtained an active aqueous Hisaw extract and an active lipoid female sex hormone extract from the same batch of corpus luteum.

Nomenclature

The utmost confusion prevails in the nomenclature applied to the female sex hormone and substances giving this reaction. This is largely due to the fact that the active substance has not been isolated and that investigators have been inclined to name the active fractions according to the tissues from which derived.

Therefore, the earliest investigators called the supposedly active substance "ovarin" (Poehl), "oöphorin" (Landau), "biovar, protovar, luteovar" (Okinschitz) which all refer to the derivation. On the other hand, Seitz, Wintz, and Fingerhut (414) called their product "sistomensin," believing that this influenced the menses to subside, and "agomensin," a fraction which was to stimulate the menstrual flow. Schickele, Aschner, Frank, and others were content to call their active fraction "corpus luteum" containing, or "placenta" containing hormone, and later, in 1917, Frank spoke of "follicle fluid" as such. As early as 1911, G. Klein (253a) used the word "folliculin" which has been taken up by others.

“Feminin” (Glimm and Wadehn, 184), “gynacin” have likewise been applied to an active fraction. In 1923 Allen and Doisy spoke of an “ovarian” hormone, “follicular” or “estrous” hormone, all applied to the same active substance. Loewe of Dorpat likewise used the word “estrous hormone.” Frank and Gustavson, on the other hand, in order not to prejudice the source and derivation of the hormone, have used the noncommittal term of “*female sex hormone*” which since has been used by many investigators. From 1925 to 1926 on, Zondek and Aschheim have used the term “ovarian hormone.” Parkes and Bellerby speak of “oestrin” whether derived from follicle or placental material.

Laqueur calls an active fraction which is water soluble and contains not more than .01 mgm. of solids per mouse unit, “menformon.”

Steinach and others have spoken of the “cyclical” hormone (Hormon des Geschlechtszyklus).

For those who care to use Greek nomenclature, sufficiently inclusive terms have been proposed by Loewe and by Poll.

Loewe, in 1926 suggests the inclusive nomenclature of thelykinin (θῆλυς κινέω) “thelys” signifying “the feminine,” and “kineo” I set going. As sub-titles he gives “thelystasin,” the inhibiting portion, and “thelytropin,” a combination. For the male hormone he suggests the name of “Androkynin.”

Blotevogel, Dohrn, and Poll suggest the name of “tokokinin” which signifies the procreative hormone (Zeugungshormon) applicable to both male and female.

I really feel that the term female sex hormone covers all needs until we know more about the substance itself, as it embraces the entire activities, not prejudicing the derivation from any source. The term is applicable to any substance which either increases or actually establishes feminine characteristics and feminineness. The innumerable trade names now applied to supposedly active fractions will be taken up later (see page 267).

CHAPTER VII

CHEMISTRY

THE investigation of the chemistry of the female sex hormone conveniently may be divided into three different periods. The first and earliest period was one in which but little information was gained from aqueous extracts and press juices. In the second, the most fertile period, the lipoid soluble substances were investigated. At present we witness the beginning of a third period, in which water soluble and very active fractions are being studied.

I. Early Press Juice Experiments

The work of Ott and Scott in 1914 (352), Niklas (347), Villemin (455), etc. was mainly focused on acute, almost instantaneous effects from ovarian, fetal, or placental aqueous or saline extracts. Marked effects on the blood pressure, high general toxicity, showing itself in convulsions and death were noted. These effects were correctly interpreted by Frank (134) as resulting from intravascular clotting (see page 57).

Ott and Scott (352) worked with aqueous extracts as early as 1902. They gave aqueous extracts of corpora lutea to virgin rabbits, obtaining breast changes. No chemistry.

Niklas (347) using placental and fetal tissues made by sodium chloride aqueous extraction from the alcoholic precipitate of aqueous extracts of these organs, merely studied the galactogogue effect. No chemistry.

Villemin (455) injected aqueous corpus luteum extracts intravenously with marked circulatory depression and death preceded by convulsions.

Frank (134) proved that intravascular clotting produced the convulsions and death. He prevented these phenomena by a preliminary hirudin injection.

Frank's results were confirmed by Adler, 1912 (2) who also used hirudin with the same effect.

Biedl, 1926 (42, page 382) apparently misinterpreted my conclusions. He discusses my experiments with those of investigators who judged of the toxicity by results obtained from intravenous injections. My experiments were the same, my conclusions diametrically opposite.

II. Lipoids and Lipoid Soluble Fractions

Dating from the pioneer work of Iscovesco (230–239) from 1908–14 a real advance was made. He found that by means of lipoid solvents, marked concentration of the active fraction could be obtained. Iscovesco, by his technic, was able to remove much of the protein, most of the neutral fats, considerable of the cholesterol and other impurities. Yet, as he clearly realized, his final product was extremely impure. In brief outline, his method of procedure was as follows.

> 1. The organ was pulped and for 2 hours kept in 3 times its volume of 95 per cent alcohol.
>
> The alcohol soluble formed *fraction I.*
>
> 2. The residue was dried at 40°C. and Soxhleted for 40 hours with neutral ether or with petroleum ether. Ether soluble =*fraction II.*
>
> 3. The residue dried, extracted with acetone. Acetone soluble =*fraction III.*
>
> 4. The residue was dried, extracted with chloroform. Chloroform soluble =*fraction IV.*
>
> 5. The residue was dried, again extracted with alcohol. Alcohol soluble =*fraction V.*
>
> Each of these groups (I–V) was taken up in turn and refractioned.
>
> Let us follow group II which contained the utero-stimulant portion of the ovary and placenta.
>
> *Group II* (*Ether soluble fraction*).
>
> Ether residue again taken up in ether.
>
> Two fractions resulted (II)$\begin{cases}\text{Insoluble (A)}\\ \text{Soluble (B)}\end{cases}$
>
> II B dried; dissolved in 10 volumes of acetone;
>
> ice box 10 hours $\begin{cases}\text{Precipitate (C)}\\ \text{Mother liquor.}\end{cases}$
>
> Mother liquor concentrated; ice box, a yellow-white mass frozen out (D).
>
> Filtrate dried, dissolved in hot alcohol. Cooled 24 hours
>
> $\begin{cases}\text{Soluble (E)}\\ \text{Insoluble (F). (F mainly cholesterol).}\end{cases}$
>
> Refractioning of each group gave further subfractions obtained with
>
> a. ether
> b. cold $\left.\begin{matrix}\\ \\ \end{matrix}\right\}$ alcohol.
> c. hot
>
> The utero-stimulant fraction by this method proved to be II Ea.

Herrmann, in 1913 and 1915 (217–8) advanced the purification considerably farther. In 1913 his end product was supposedly an unsaturated penta-amine-di-phosphatide. This final product proved to be a fluorescent oil which solidified on cooling, gave a strong cholesterol reaction, became brown in the air from the absorption of oxygen. This final product contained merely carbon, hydrogen, and oxygen. It was soluble in ether, petroleum ether, acetone, benzene, and was insoluble in water.

> Using part of the technic described by Iscovesco, Herrmann reduced both the entire ovaries and the corpus luteum alone to a dry powder (vacuum drying at 37°). This powder was Soxhleted with ether, re-extracted with acetone, then with ether and chloroform. These fractions were united and finally extracted with methyl alcohol.

> He then froze out the cholesterol and repeated the ether extraction. He then distilled in vacuo at an internal temperature of 193°C., the vacuum being .06 mm. mercury. His final product still gave a strong cholesterol reaction; carbon 81.33 to 81.62 per cent, hydrogen 11.32 to 11.49 per cent. In his opinion the pure substance was a cholesterol derivative.

Frank and Rosenbloom in 1915 (165) expressed their inability to determine whether the active substance was a lipoid or merely carried along by the lipoids. But because the extracts containing all the fat soluble substances were found to be by far more potent than any of the fractions derived from them, (elective solubility in alcohol, ether, acetone, or chloroform) they decided that it appeared more probable that the active substance is not a lipoid but is carried along by the lipoids.

In 1917 Frank (141) summarized the result of investigations conducted with Dr. J. Rosenbloom, Mr. O. I. Lee and P. M. Giesy as". . . . the potent crude extract, which is obtained by continuous extraction with alcohol, is a dark, semi-liquid mixture which resists high degrees of heat (350°C.), strong acids or alkalies and saponification without loss of potency. It either oxydizes or polymerizes on standing, gradually losing its activity. It is fully soluble in 95 per cent alcohol, partially so in chloroform, ether, oils, and in benzin (the active fraction at times following the benzin insoluble portion). *The activity persists after removal of cholesterin and indol,** which may be regarded as contaminations. The active substance has not been sufficiently purified to permit of analysis. Our attempts to duplicate Herrmann's work lead us to conclude "that his final product was a mixture and not a single compound."

The main finding to be emphasized in this last investigation is the fact that the active substance could be freed from cholesterol and that it resisted the action of strong acids and alkalies, and could be saponified with impunity.

Giesy in 1920 published a dissertation for his doctor's thesis (182), much of which was done under my direction. Some of the later work in this thesis, based upon assays and titers performed with the wet uterus weight compared with the body weight, should be received with caution unless verified by other methods, as this type of assay is entirely unreliable as I have discussed on page 85.

The main results Giesy obtained may be summarized as follows.

> The substance under investigation was found to be soluble, under definitely described conditions, in benzene, chloroform, carbon tetrachloride, absolute and 95 per cent alcohol, ether, ethyl acetate, carbon disulfide, pyridine, acetone, acetyl chloride, and hot acetic anhydride. It was somewhat soluble in 50 per cent alcohol and glacial acetic acid. Under certain conditions, it was soluble in water and benzin (petroleum ether).

---

* Indol was found, if large amounts of human placental material were not at once minced and thoroughly mixed with alcohol, probably due to an autolytic change.

> With water and benzene, no active substance was found in the aqueous layer, while the benzene layer was active. With water and benzin, the distribution depended on the other substances present. *With benzin (petroleum ether) and 75 per cent alcohol, most of the active substance was present in the alcoholic phase.*
>
> With rubber membranes, the substance dialyzed in acetone, benzene, ether, and chloroform solution, and dialyzed slightly in benzin and carbon tetrachloride solution. With collodion membranes, the substance dialysed in 62.5 per cent and 75 per cent alcohol solution, but not in 50 per cent alcohol solution.
>
> The activity was not destroyed by heat, except in the presence of air. Alcoholic solutions lost activity on standing; benzene solutions did not. The activity was slowly destroyed by acids, alkalis, and bromine, and rapidly by nitrous acid and cold alkaline permanganate. It was not destroyed by acetyl chloride, boiling acetic anhydride, zinc and glacial acetic acid, aqueous sodium carbonate, sodium bisulfite, cadmium chloride, fuller's earth, phenylhydrazine, hydrocyanic acid, hydroxylamine, or semi-carbazide.
>
> A concentrated preparation of the substance contained neither nitrogen, sulfur, nor halogens. The tentative conclusion has been reached that the active substance is probably an unsaturated compound of ester-like nature, containing neither the carbonyl, carboxyl, amino, nor hydroxyl grouping.

For the purpose of concentration, Giesy suggested a partition between benzin (petroleum ether) and 75 per cent alcohol, using the method after the manner of fractional crystallization, a slight modification of which method is now being used by Allen and Doisy and their co-workers. *Giesy's priority in the publication of this method should be recognized.*

The main criticism of Giesy's chemical research is the method of assaying which he developed and which, in my opinion, is quite unreliable. In spite of this handicap, where strong positive reactions were obtained, Giesy's results may be accepted. He appears to have been aware of the weakness in his titration by the weighing test.

> "The present method appears to have two advantages over that of Frank: it is more rapid, and its results are expressed in terms of an objective standard, so that the results obtained with it by different experimenters can be compared. It is possible however, that Frank's method, on account of the integrating effect of using four injections, may give more uniform reactions than the present one."
>
> The method consisted in a single injection of 25 mg. irrespective of the substance or of the weight of the animal, into young rabbits varying between 300 and 600 grams. The animal was killed 4 days later and the uterus excised, dried in vacuo over sulphuric acid, and weighed dry. Empty body weight (that is after removal of the intestinal tract) was then compared with the dried uterine weight. Giesy's controls numbering approximately 28 with empty body weights varying between 100 and 700 grams appear to be entirely insufficient.

The next article of considerable importance was that of Fränkel and Fonda, 1923 (167) who, continuing the work of Herrmann, described a somewhat related method of extraction which embodies Iscovesco's and Herrmann's technic and may be briefly summarized as follows:

> Starting with corpus luteum and placentae, the tissues were
> 1. Ground, dried *in vacuo* at 37°.
> 2. Extracted with ether.
> 3. Ether residue triturated with acetone.
> 4. Cholesterol removed by chilling (mother liquor treated with 96 per cent alcohol and again chilled). Filtered.
> 5. Concentrated filtrates taken up with methyl alcohol and chilled to remove neutral fats.
> 6. Evaporated down and distilled in high vacuum.

Their final product, like that of all predecessors, was a viscous, yellowish oil which still gave a strong Salkowski Liebermann-Burchard reaction (cholesterol). The oil proved optically inactive as could have been predicted from the extreme dilution of the substance, even should it later prove to be optically active. They gave their "pure" substance the empiric formula of $C_{32}H_{52}O_2$. In addition they prepared a tetrabromide, a phenyl hydrazone, an oxime, a benzoate, and a barium compound. This ambitious work would have been of much value if these chemists had checked up their fractions biologically. They failed to do this throughout, as the repetition of their investigations has convinced us (Frank and Gustavson 163):

> Practically all of the claims made by Fränkel and Fonda, except such as had been previously demonstrated to be correct by Herrmann (218), Frank (141), and Giesy (182), have been proved to be wrong. The "final product," the same as that of their predecessors, was again a mixture and not a pure substance.

In 1924, Doisy, Allen, Ralls, and Johnston (93) starting out with follicle fluid, described methods of obtaining more concentrated preparations which, however, were in no way chemically purer than those obtained by their predecessors.

They removed cholesterol by the digitonin method with full retention of potency, thus confirming the results of Frank (141) obtained in 1917.

> Their then routine method of obtaining a concentrate from the fresh liquor folliculi consists in removal of protein by means of 95 per cent alcohol. The dry residue is emulsified with water and again taken up with alcohol to which, on boiling, two volumes of acetone are added. These leachings remove the hormone from the remaining solids (mainly phospholipins and inorganic salts). The alcoholic residue is again leached with small quantities of boiling 95 per cent alcohol, then cooled in an ice salt mixture, leaving behind fatty material. The alcohol residue is taken up with anhydrous ether and is kept as a stock solution from which, as needed, the ether is driven off and the residue taken up in Mazola oil (purified corn oil).

This method of preparation resembles the original technic of Iscovesco more than that of Herrmann, who relied on vacuum distillation to a considerable degree.

In a later article appearing simultaneously with an article of our own (Frank and Gustavson, 163) and simultaneously presented at a meeting of the American Association for the Study of Internal Secretions, Doisy, Ralls, and Jordan (94) reported the concentration of the active principle with considerable modifications from their first described method.

> Follicle fluid was, as previously, treated with 95 per cent alcohol, 2 volumes. The alcohol residue was taken up in water and extracted with ether. The ether residue was taken up in 70 per cent alcohol and five times in succession treated with petroleum ether (benzin), because it was found that cholesterol is 29 times more soluble in petroleum ether than in 70 per cent alcohol. The material thus obtained gave 1 R.U. to each 0.04 mgm., which exceeded the minimum demanded by Laqueur (1 M.U. equals 0.1 mgm.). The composition of their purest product was carbon, 80.8 per cent; hydrogen, 10.5 per cent; nitrogen, 0.93 per cent; phosphorous, 0 per cent, (by Pregel microanalysis). They gave a molecular weight of 458. It is quite evident from the presence of nitrogen, which it might be added, would necessarily require a molecular weight of at least 1505 ($0.93:100=14:X=1505$) that the end product was not as pure as that obtained by Herrmann (218), Frank (141), Giesy (182), or Fränkel and Fonda (167).
>
> Throughout, their chemical work, performed on follicle fluid, confirms the results obtained by their predecessors, including the biological evidence that the potency of the active substance is not affected by sodium ethylate, hydrogenation, phenyl-hydrazene, all of which had been previously reported by Frank and Rosenbloom (165), Frank (141), Giesy (182). In substantiation of Giesy, they also found that complete loss of potency was produced by treatment with acetyl chloride, a moderate loss by benzoyl chloride, as well as by naphthylisocyanate.

In a later and most important paper, Ralls, Jordan, and Doisy (382) published an improved procedure for the extraction of the "ovarian hormone." Their main efforts appear to have been directed toward determining a method for obtaining the greatest possible yield. In substance, their procedure is as follows:

> 1. Liquor folliculi is mixed with 2 volumes 95 per cent alcohol to precipitate the proteins. The filtered-off precipitate is re-extracted with alcohol (6 to 8 hours in Soxhlet).
> 2. Combine filtrates, and distill to dryness *in vacuo*.
> 3. The residue is hydrolyzed for a short time with either alcohol or water and gentle heat, and then dissolved in 150 c.c. of water, the entire process being gauged on the basis of one liter of liquor folliculi as a starting point. Centrifuge, retaining clear fluid.
> 4. Alkalinize with sodium hydrate so that a few drops added to a few drops of alcohol will just redden phenolphthalein.

5. Divide aqueous solution between two separating funnels, adding 30 c.c of ether to each fraction. Shake well. Centrifuge. Run aqueous solution into another funnel, repeating the ether extraction 3 to 4 times.
6. Wash combined extracts with water.
7. Distill off ether.
8. Ether residue dissolved in petroleum ether (benzin) and alcohol alternately, keeping record of volumes used and transfer to separating funnel. Enough alcohol and water are added to give a volume of 100 c.c. of 70 per cent alcohol, the quantity of petroleum ether being made up to 25 c.c.
9. The alcoholic solution is extracted 6 times with 25 c.c. portions of petroleum ether.
10. Distill alcoholic solution to dryness *in vacuo*.
11. Residue is leached with redistilled dry ether. Ethereal extract centrifuged to remove insoluble solids.
12. This ethereal solution is used as stock solution. Such extracts contain 0.03 to 0.04 mg. of solids per R.U.

Their method of removing the cholesterol is based upon Giesy's (1920) partition between benzin (petroleum ether) and dilute alcohol, the cholesterol being 26 times more soluble in petroleum ether than in dilute alcohol.

Their chemical study, although thorough and elaborate, in the main presents nothing new.

The authors emphasize the staggering cost of female sex hormone derived from follicle fluid, as but 100 c.c. of liquor can be obtained from 1000 hogs' ovaries, which makes the cost approximately $1.00 per mg.

From their careful quantitative experiments they believe that the thermostability is not absolute and that much activity is lost in distillation.

Faust (111) like Fränkel and Fonda (167) considered the active substance a cholesterin derivative. His article contains nothing of real value.

Glimm and Wadehn, 1925 (183) in general followed the technic advocated by Herrmann, and Fränkel and Herrmann. They mixed the "crude syrup" with dilute alkali. The milk-like emulsion resulting was then precipitated with lead acetate, a method which Gustavson found of some value in obtaining concentration.

In a similar contribution a year later, these same authors 1926 (184) now using the M.U. for their assay, obtained a sufficiently concentrated fraction to give 1 M.U. per 0.1 to 0.2 mg. Their method was extremely complicated and consisted in variations in fractional precipitations, such as advocated by Iscovesco, but added nothing new.

Hartmann's article in 1926 (211) adds little to our knowledge. He saponified Fränkel and Fonda's product, finding that the activity followed the neutral body, the mixture of higher unsaturated fat bodies proving inert physiologically. His product boiled at 145° in high vacuo, was unsaturated, and contained carbon, 81.65 to 82 per cent, hydrogen, 11.11 to 11.75 per cent.

Hartmann and Isler (212) to the distilling process of Herrmann added acetylization with acetic acid anhydride. Their final product was a neutral body, found physiologically active. It contained carbon, 82, 81.96, 81.65 per cent; hydrogen, 11.17, 11.28, 11.75 per cent.

In 1925 Gustavson, (195) in a dissertation for doctor's degree, summarized some of our chemical work done up to that period.

> An important contribution was a new method to remove the cholesterol by means of liquid ammonia, based on the discovery of Franklin and Kraus (170). Gustavson has detailed at a later date, together with Goodman, (196) the solubility of non-radiated cholesterol in liquid ammonia.
>
> By this new method we were able readily to free the hormone from the contaminating cholesterol in a quantitative fashion.
>
> Among the new facts established were that the hormone is not precipitated by mercurous nitrate, as we had previously supposed, though often partly adsorbed by the precipitate.
>
> Adsorption experiments were made with various precipitates. Some adsorption also occurred with phosphatide-cadmium chloride precipitate. A much superior result was obtained with lead acetate as the following experiment will show—
>
> 0.5 gram of placental stock "B" was dissolved in 10 c.c. of 95 per cent alcohol and a saturated solution of lead acetate in 95 per cent ethyl alcohol added and the solution allowed to stand over night. The precipitate was removed by filtration and suspended in 50 per cent ethyl alcohol and saturated with hydrogen sulphide. The resulting mixture was then diluted and extracted twice with benzene. 11 mg. of residue were obtained upon evaporating the benzene. The assay showed plus 4 when 7.5 mg. were injected. The experiment was so successful that it was repeated and four rats injected with the following results:
>
> Rat No. 1 injected with 3.75 mg. vaginal smear plus 4
> Rat No. 2 injected with 3.75 mg. vaginal smear plus 4
> Rat No. 3 injected with 2.5 mg. vaginal smear plus 4
> Rat No. 4 injected with 1.0 mg. vaginal smear negative.
>
> He detailed our investigation dealing with the content of female sex hormone in the corpus luteum and its variation, which depended upon the time elapsing after ovulation had taken place. Corpora lutea obtained from 4900 grams of ovaries from freshly slaughtered hogs were separated into classes corresponding to early vascularized, later vascularized, flourishing, and involuting; or, more simply, into bloody, bloody pink, pinkish yellow, and yellow.
>
> Samples were taken and analyzed with the following results:

	Bloody	Bloody Pink	Pinkish Yellow	Yellow
Grams of each class	98	484	378	69
Moisture per cent	81	79.9	79.1	75.5
Crude lipoid (per cent of dry substance)	18.6	25.4	36.6	32.2

Nitrogen (per cent of dry substance)	12.6	10.68	10.45	10.38
Phosphorus (as per cent of pentoxide of dry substance)	2.68	3.38	3.44	2.42
Ash (per cent of dry substance)	4.63	5.26	4.92	3.95

> Stock B (crude alcoholic extract, see page 141) was then prepared from each of these classes and suspended in olive oil with a dosage of 50 mg. per c.c. Assayed by the vaginal smear test on rats the results were

	Number of	Total	Reactions	
Class	injections	dosage	1st animal	2nd animal
Bloody	3	150 mg.	negative	negative
Bloody-pink	3	150 mg.	negative	negative
Pinkish-yellow	3	150 mg.	plus 1	plus 1
Yellow	3	150 mg.	plus 3	plus 2

> The striking result is the concentration of the active material in the yellow class.

These experiments were repeated, varied and amplified on many occasions. As I stated (Frank, 148) in 1926, until we divided the corpora lutea into definite classes, depending upon the time elapsed since ovulation, *47 batches tested proved negative and only 27 positive.* Since this selection was adhered to, the last ten batches all gave positive results. With as little as 28 mg. of corpus luteum lipoid, a positive rat reaction could be obtained. Comparing, roughly, the minimum amount of solid per R.U. it was found that of the crude extract

Follicle fluid lipoid	15 mg.
Placenta lipoid	37 mg.
Corpus luteum lipoid	75 mg.

In 1926, Frank, Gustavson, Holloway, Hyndman, Kreuger, and White (163) gave a summary of further chemical work.

> This dealt especially with the evidence showing the presence of an active fraction in the corpus luteum (for details of which see Gustavson above), which was discussed from the physiological standpoint on page 103.
>
> Emphasis was placed upon the fact that, whatever chemical qualities characterized follicle fluid, applied equally to corpus luteum and placental extracts.
>
> The solubility of the active principle in water was stressed, but limited to certain conditions, depending on the presence of other extractives and other methods of purification.
>
> While Herrmann (218) and Frank and Lee (141) had obtained distillation of the active substance in high vacuum between 190° and 250° without loss of potency, further purification obtained by us

enabled us to distill the active substance at 30 mm. *in vacuo* at as low a temperature as 100°.

The fact that the active substance could be extracted from alkaline solution with ether favored the absence of any acid group; a similar extraction from acid solution might signify the absence of a basic group. Giesy has previously shown that no loss of activity occurred by digestion with soy bean, which favored the absence of an ester.

The previous saponification experiments had been amplified by saponifying with potassium hydroxide, sodium methylate, sodium butylate, and sodium ethylate for as long as 72 hours, without loss of potency.

This signifies that the active principle is either not saponifiable, or if it is an ester, the activity must reside in the alcohol of high molecular weight liberated by the saponification process. This disagrees with Dickens, Dodds, and Wright (91) who found the hormone slowly destroyed by saponification.

We also disagreed with the conclusions of Fränkel and Fonda who had claimed that they had succeeded in obtaining an active phenyl hydrazone.

We likewise succeeded in obtaining a phenyl hydrazene compound from the lipoid fraction of placenta, but the mother liquor, after removal of the phenyl hydrazene compound and excess of phenyl hydrazene remained active physiologically. Moreover, when the phenyl hydrazene compound was decomposed by being allowed to react with glucose, the lipoid liberated was found to be physiologically inactive. Fränkel and Fonda had failed to perform this necessary control.

At that time it seemed that the active substance was an unsaturated body which absorbed bromine from a carbon tetrachloride solution. Some evidence to the contrary has since then been obtained by us.

The preparation adsorbs bromine. We did not succeed in testing the brominated product to determine whether it was active, as it proved quickly lethal to the test animals.

The ready oxidation of the active substance has been known for years, with the resultant physiological inactivation. While PCl_5 always does, PCl_3 does not affect the hormone. The inactivation by PCl_5 may be due to oxidation, or replacement of hydroxyl or carboxyl group by chlorine.

The fact that PCl_3 does not destroy the activity would indicate either that the hormone does not contain a free hydroxyl group or that replacement of the OH by Cl has no effect on the activity. Halogens are found in our lipoid preparation only after PCl_3 or PCl_5 treatment. The conclusions are summarized:

The active substance is a thermostable substance acting like a lipoid. It appears to be of high molecular weight, yet its great thermostability must be contrasted with this assumption. It is soluble in all lipoid solvents and also in water under certain conditions. It contains only carbon, hydrogen, and oxygen, and possibly merely carbon and hydrogen. It is not an ester nor a fatty acid. It does not

> give cholesterol reactions. It does not contain an active carbonyl group. If it contains a free hydroxyl group, this group is not essential to its activity. It is an unsaturated compound and is inactivated by attacking the double or triple bonds.

Our own efforts (Frank and Gustavson, largely unpublished) during the last three years have centered on the purification and chemical identification of the active substance regardless of any loss incurred during the purification. Although we have prepared and assayed biologically more than 1000 individual fractions during this time, comparatively little progress can be recorded. After all, this should not be surprising when we reflect that although numberless chemists during the last 10 years have concentrated on this problem, the results obtained by Herrmann in 1915 and amplified by Frank in 1917, have remained unchanged and almost unexpanded.

> A study by means of the spectroscope in the ultra-violet portion of the field, conducted by Gustavson in Chicago during the year 1927, showed absence of any characteristic absorption bands.
>
> There was a general absorption but nothing specific. This applied equally to material obtained from follicle fluid, corpus luteum, and placenta.
>
> Evidence in favor of the presence of an aldehyde group has been collected. The fact that the activity is readily destroyed by oxidation is one characteristic of many compounds, especially aldehyde, ketone, and double bonds. The substance is reduced by hydrogen in the presence of colloidal platinum as catalyst, using the method of Roger Adams. This effect might be produced by either the presence of an aldehyde, ketone, or double bond, but we find that weak oxidizing agents appear to restore the activity which is interpreted as a re-oxidation of the alcohol back to the aldehyde. Presumably the alcohol is inactive. Too strong oxydizing agents apparently oxydize the substance to an acid with no restoration of activity.
>
> The destruction of the hormone by oxidation may mean that the aldehyde is oxidized to an acid. The inert oxidized product may be reactivated by reduction with hydrogen, using platinum oxide as a catalyst. It is a well known fact that simple acids cannot be reduced to aldehydes. If the hypothetical aldehyde contained a hydroxyl group in the gamma position, the acid formed on oxidation would form a gamma lactone and these lactones are easily reduced back to aldehydes. The experimental facts can be explained on the assumption that we are dealing with a gamma hydroxy aldehyde.
>
> When treated with sodium acid sulphite the hormone gives a certain titration with iodine. A similar amount of sodium acid sulphite gives a higher titre, that is a typical aldehyde reaction and would be conclusive if the presence of impurities could be entirely ruled out.

Our latest evidence then, of which only this small portion has been presented, but which we expect to publish *in extenso* shortly, makes us believe that the hormone is the lactone form of a gamma hydroxy aldehyde.

Detection of a double bond was attempted by using iodine, employing Hubl's method, (iodine chloride), Hanus' method (iodine bromide) and Wiji's method (iodine glacial acetic acid). No evidence favoring a double bond was found, i.e. absorption of iodine takes place, but the activity of the hormone is not destroyed.

A final effort to detect or exclude the free hydroxyl group was made by dimethyl sulphate which forms an ether and therefore would allow of no possibility of hydrolyzing in the body, back to an active compound. With acetic chloride, benzyl chloride and even phosphorus trichloride (PCl_3) esters might be formed which conceivably might hydrolyze in the test animals and regenerate the hydroxyl group and thus restore activity. The treatment of active preparations with dimethyl sulphate does not destroy the activity. The lactone form previously referred to would not be attacked by dimethyl sulphate.

The fact that saponification did not destroy the activity cannot be taken as positive evidence that there is no ester in the molecule. The activity might conceivably reside in the alcoholic fraction of the ester and the activity depend on the ester linkage. This question has never been entirely answered to our satisfaction.

A large amount of effort has been expended by us (Gustavson in Denver, Frank, Swobotka, and Goldberger in New York) in trying to duplicate the results obtained by those investigators who report active water soluble fractions. The work of Zondek and Brahn, Zondek and Aschheim, Laqueur *et alia*, Dickens, Dodds, and Brinkworth, to mention only some, has been repeated by us as well as the descriptions given by these authors allowed us to. On the whole, the results have been extremely disappointing.

> With the Zondek-Brahn-Aschheim technic only very weak fractions could be obtained. Laqueur's confusing descriptions have made it impossible for us to duplicate his work in any way. A number of repetitions of Dickens and Dodds experiments have given us a final product which showed no activity whatever.
>
> As yet we have been able to obtain our best fractions by simple extraction of pregnancy urines with ether, the ether residue proving more or less soluble in water. As will be mentioned further on (page 144) there is not only a tremendous loss of hormone during purification but also a rapid loss of activity of the hormone when kept in aqueous solution.

During these years much time has been expended in obtaining fractions suitable for physiological experimentation and for human injection. Such suitable material has only been obtained in oily media which has prevented us from utilizing it on a large scale clinically.

Our active substance is described under the following titles:

Stock A is the crude alcohol extract of follicle fluid, corpus luteum, or placenta.

Stock B is the alcoholic residue extracted with benzene.

Stock C is Stock B extracted with 70 per cent alcohol. Stock B, dissolved in 70 per cent alcohol and fractionated with petroleum ether, according to the method of Giesy, the hormone remaining in the alcohol.

Stock D, the ether extract of Stock C prepared with anhydrous ether and extracted with liquid ammonia. Stock D = the ammonia soluble fraction.

The comparative potency of these extracts as well as the huge losses entailed in our method of purification, are best shown by an illustrative experiment.

Starting with a given batch of placenta of 15 Kg. weight

	Total	*Total R.U.*
Stock A = 1 R.U. per 70 mg.		
Stock B = 1 R.U. per 37 mg.	20 gm.	540
Stock C = 1 R.U. per 8 mg.	2 gm.	250
Stock D = 1 R.U. per 2 mg.	0.2 gm.	100

We are unable to account for the large amount of ballast, or impurity, carried along with our present end product to account for the difference in weight per R.U. and that of Doisy (382) for example (as 2 mg. :0.03 or 0.04 mg.) or that of Doisy with that of Lipschütz (294) (as 0.03 or 0.04 mg. :0.00008 mg.)

III. Water Soluble Hormone

As early as 1915 Frank and Rosenbloom (165) considered it probable "that the active substance is not a lipoid but is carried along by lipoids."

Zondek in 1923 (480) investigated the water soluble extracts then on the market and showed that none possessed any activity.

In 1925, Zondek and Brahn (491) obtained an active water soluble preparation. A brief description is also given by Zondek. Their technic consisted in the following.

> 1. The raw material (follicle fluid or placenta) is treated with 96 per cent alcohol, kept several days in an incubator. The precipitated proteins are further extracted with alcohol.
> 2. The alcohol portions are united and fully saponified with strong alkali.
> 3. The alcohol is distilled off, the soaps dissolved in water.
> 4. Shaken with ether, retaining ethereal portion.
> 5. The ethereal residue (a white powder) is taken up in alcohol.
> 6. The alcohol is heated and acidified with dilute acid.
> 7. Filtration and neutralization give a water clear solution containing the hormone.

In 1925, Loewe (305) discovered female sex hormone in the urine of cyclical women. Influenced by this, Zondek, in 1928 (482) described the recovery of the female sex hormone from the urine of pregnant women in which it occurs according to him in the high concentration of from 4 to 6,000 M.U. per liter. Zondek therefore advocates this source as a starting point of the extract. His technic is as follows:

> (1) The "purified" urine (probably acidified with acetic acid and then filtered) is extracted with a lipoid solvent, which should not be alcohol, as this dissolves urea. Therefore we use ether for this extraction.
>
> (2) The ether residue is taken up in water and treated with hot alkali.
>
> (3) The water solution is shaken out with ether.
>
> (4) The ether residue is taken up in distilled water or in hot dilute acetic acid and filtered.
>
> (5) Phases 3 and 4 may be repeated several times for further purification.

The filtrate is said to contain the hormone in a water-clear, colorless and odorless solution. A solid residue of 0.01 to 0.001 mgm. is found per M.U.

This extraction for female sex hormone may profitably be combined with a simultaneous recovery of the extract of the anterior lobe of the hypophysis which also has been found in the urine of pregnant women by Zondek and Aschheim (489). The only modification necessary is that step (1) is carried out in the following fashion.

> (1) Acidify the urine slightly with acetic acid and filter.
>
> (2) Concentrate *in vacuo* at a temperature not above 40°C. (as the anterior lobe hormone is thermo-sensitive) to one half the amount.
>
> (3) Filter, removing sediment.
>
> (4) Then extract with large amounts of ether, the process then being continued as with the ether residue, step (2).
>
> The water which contains the anterior lobe hormone is then treated according to the further procedure outlined by the authors.
>
> The Zondek water soluble female sex hormone is marketed under the name of "Folliculin."

From 1925 on, Laqueur and his group (272–3) likewise reported on the production of a water soluble female sex hormone which they called "menformon." These authors detailed a confusing variety of optional methods.

> They suggested removal of the protein with such diverse agents as colloidal ferric hydroxide, chloroform, or other lipoid solvents, but I confess agreement with Dickens and Dodds as to my inability to follow their technic as detailed.

In a later publication, Laqueur (270) takes exception to the fact that Dickens, Dodd, and Brinkworth (89) were unable to obtain the hormone by the method previously described. However, in this new publication, Laqueur gives the following directions with placenta as the raw material:

> 1. The placenta is used as fresh as possible.
>
> 2. It is finely minced.
>
> 3. It is boiled with one and a half times its volume of benzene for at most one hour.

> 4. The benzene is filtered off as quickly as possible and on evaporating the fluid a yellow residue remains, weighing between 200 and 300 mgm per kg. placenta. It contains about 1000 mouse units (M.U.) This is only about one-third of the total amount present in human placenta. On treating it with water, sometimes part of the hormone dissolves to a clear solution, but this is by no means regularly the case, and often the fluid is turbid. It is therefore better to proceed as follows:
>
> 5. The residue is repeatedly triturated with small amounts of 80 per cent alcohol (usually altogether about 50 c.c.) until the alcoholic layer after centrifugalising is quite colourless. Thus a large part of the impurities are left behind.
>
> 6. The alcohol is evaporated; the weight of the remaining residue is about half that of the first crude product.
>
> 7. The residue is shaken with 500 c.c. of water and 1.5 to 2 c.c. of 25 per cent HCl for about half an hour.
>
> 8. It is then left at a temperature of +2° for about 12 hours.
>
> 9. Then it is centrifugalised or filtered through glass wool.
>
> 10. Finally, if the fluid is not yet quite clear, it is filtered through asbestos (long fibres, treated wit decinormal HCl and washed with water.) Common filter paper should be avoided because it adsorbs large quantities of active material.
>
> The solution thus prepared contains as a rule about 300–400 M.U. The output may be increased to about 600 M.U. by extracting glass wool and asbestos with benzene, evaporating the solvent and dissolving the residue in water in the manner just described.

Laqueur then mentions elective variations in his method. For example, by autoclaving the placenta in water, it dissolves completely and may be utilized, instead of step (2) of his detailed technic.

In step (5) he says that shaking the 80 per cent alcoholic solution with "activated" charcoal and then freeing the adsorbed hormone with phenol may be tried. The phenol is then removed by distilling with steam and the residue re-dissolved with benzene. The end products thus obtained have a minimum activity of 1 M.U. per 0.1 mg. of residue, but by repeatedly shaking the watery solution out with benzene, further purification to 0.0008 mg. per M.U. has been reached.

The chemical qualities of his menformon were summarized as follows (263):

> The purest substance gave no cholesterol reaction nor did it contain sulphur or phosphorous, and probably no nitrogen. No characteristic groups (radicals) could be isolated. Marked thermostability, toleration of 230° at 40 atmospheres pressure, even in water. No deterioration at 190° with 25 per cent sulphuric acid or boiling with 20 per cent sodium hydrate.
>
> The active substance resisted pancreatic enzyme as well as pepsin.
>
> However, in conformity with all the other investigators, marked susceptibility to oxidation was found.

The similarity of these qualities with those found by previous investigators who worked with lipoid soluble fractions is self-evident. Laqueur's product is marketed under the name of Menformon.

Water soluble preparations have also been described by Lipschütz *et alia* (294) who by using the "routine method" of Allen and Doisy, adding saponification to this and extracting with acetone (aqueous) obtained an active fraction containing 1 M.U. to 0.015 mg. dry substance. By repeated water to ether extractions a substance active to 0.00002 mg. was obtained which equals 0.2 c.c. of a solution of 1 to 10,000,000 per M.U. *These aqueous solutions lose all activity in the course of a few days.*

Biedl (43) by electro dialysis of an emulsion of ovarian lipoids, during which the active substance passes through the membrane to the negative pole, obtains a water (also ether soluble) product which is being marketed under the name of Hormovar.

Slotta, 1927 (418) extracts the female sex hormone from urine of pregnant women (no details). He believes the pure substance related to cholesterin even if it no longer gives the cholesterin reaction.

Veler and Doisy, 1928 (453) obtain from 480–1240 R.U.L. from pregnancy urine. The strongest material gave 0.06 mg. solids per R.U. Their technic is a variation of the lipoid extractions, applying the chloroform extraction which Laqueur gave as one of the elective methods.

Quite recently, Doisy, together with Thayer and Jordan (439) has published further "improved procedure" in the extraction of the ovarian hormone. The gist of this article consists in using greater precautions in order to avert the destructive effects of peroxides which may be found in ethyl ether and in petroleum ether.

In addition, the authors have reduced the R.U. below 0.001 mgm. With these dilutions water may be added to the alcoholic solution containing the hormone, the alcohol then being distilled off. Any opalescence may be removed by adding a drop of dilute hydrochloric acid and keeping the aqueous solution at 1° for a few hours. The authors justly mention that in such minute quantities it is a question whether the hormone itself is really water soluble.

Steinach and his group (430–1) obtain the active principle from the placenta of cattle- 1 c.c of aqueous solution contains 500 M.U. For biological assay he employs the nipple and breast changes in the virgin castrate albino guinea pig. This product is on the market under the name of "Progynon."

For the sake of completeness, I mention the work of Dickens, Dodds, and Brinkworth (89-90) who described a method of preparing a water soluble form of the hormone by means of an acetone picric acid process with an end product yielding one R.U. per 0.5 mg. of dry substance. "The chemical substance appears to be relatively simple and contains a fairly large proportion of sulphur. The Pauly reaction for histidine is strongly positive. Tryptophane also appears to be present."

From their own description it is evident that the end product is very impure. Our repetition of their work has given us a white powder, soluble in water, of which as much as 40 mg. (supposedly equal to 80 R.U.) did not contain a rat unit.

> When this discrepancy was called to the attention of Dr. Dodds, in a personal communication under date of March 5th, 1928, he stated that they were in process of developing a new method which will overcome these difficulties (variable adsorption by the picric acid precipitation).

Quite recently Allan, Dickens, Dodds, and Howitt (2a) have published a new method of preparing the female sex hormone in water soluble form from placenta.

> To the minced placenta, aqueous barium hydroxide is added and the mixture is boiled for two hours. The concentrated filtrate is extracted with butyl alcohol. Almost all the alcohol is distilled off, the concentrated filtrate is shaken out with ether, using four successive portions of one-half liter of ether. After some further purification the residue is dissolved in a minimum amount of alcohol and taken up with water. Ether extractions are again repeated and the ethereal extracts are washed with dilute hydrochloric acid which again has to be removed by distilled water.
>
> Through the kindness of the authors, I was supplied with a considerable amount of absolutely clear extract which, however, was found to be extremely weak. The vials were labeled 10 units to the c.c. However, titration according to the directions of the authors showed only 1 rat unit to one-half c.c.
>
> I see no reason for adopting the extremely complicated method advocated by the English authors.

Summary of Chemistry

It has proved impossible to consider every paper dealing with the chemistry of the female sex hormone. However, a serious effort has been made to analyze those communications which picture the gradual, perhaps disappointing, advances made.

It must have become apparent that, although water soluble preparations may prove of utmost value, as yet the rapid deterioration of such solutions bars their effective clinical use. This will be taken up in detail on page 273.

Although great concentration has been effected, corresponding purification has not been achieved. By this I mean that much inert ballast has been discarded by means of the newer methods of preparation. The result has been to reduce the amount of solids per M.U. to 0.1–0.001 mg. in comparison to 0.5 mg. of the lipoid soluble fractions. And yet some of these concentrated fractions appear to contain nitrogen which has been completely removed in the less "pure" preparations.

Characteristic of all of the water soluble preparations is the property of passing completely from the aqueous into the ethereal fraction at all dilutions. Whether this is an argument against the true water solubility of the hormone, I will leave to others more competent to decide.

Our repetitions, as far as has been practicable, with the incomplete descriptions furnished by Zondek and Brahn, Zondek and Aschheim, and Laqueur, have given us minute yields compared to those claimed by these investigators.

In the first place, in our starting material consisting of pregnancy urine, we rarely find more than 200-300 M.U.L. These authors find from 1000-7000 M.U.L. How to account for this huge difference in the untreated starting material is inexplicable to me, even if we allow for a large factor of variation resulting from differences in reading the end point of the mouse reaction.

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PART I

Including Nos. 1–491

Note.—For References 491a to 528 inclusive, See Part II, page 301.

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PART II

CLINICAL INVESTIGATIONS BASED ON THE FEMALE SEX HORMONE BLOOD TEST

CHAPTER VIII

THE FEMALE SEX HORMONE BLOOD TEST; TECHNIC AND APPLICATION

PART I of this monograph was devoted to the exposition of how the female sex hormone was discovered and studied. Its derivation, distribution, physiological functions, and chemistry were described as far as our present knowledge will permit.

In 1925 Loewe of Dorpat (305) and my group (154) simultaneously and independently demonstrated the female sex hormone in the circulating blood. Not long after it was demonstrated in the blood of females, it was demonstrated in the urine by Loewe (311). The strange anomalous finding of this same principle in the blood and urine of males has also been described on page 114.

Based upon these findings it was natural for me to study and investigate the human female, and Part II of this book is almost exclusively devoted to the information gained through these studies. The ground covered includes the devising of a suitable method and the establishment of norms, as a basis for further work. After these necessary facts were determined, investigation of pathological conditions was pursued, and the value of the test in differential diagnosis was studied. Its use in substituting specific in place of empirical treatment was likewise demonstrated.

These investigations were begun in the fall of 1925, in New York City, with the cooperation and aid of my co-workers, Drs. Morris A. Goldberger and Gertrude Felshin Goldberger, who have continued to work with me since then. This work, although pursued under difficulties due to the small quarters, limited assistance and funds at our disposal, has proved fruitful as I shall try to show in what follows. Our own handicaps in equipment have convinced us that the average hospital laboratory equipment and personnel is well able to perform this test as a routine just as the Wasserman test is performed in hospital laboratories.

The space at our disposal has been limited to a single room. In this there are 25 rat cages in which 250 castrated mice can be kept for the test. In addition our quarters harbor 75 rats and frequently rabbits and guinea pigs, which are used in different forms of physiological research experimentation connected with our work, but are not needed for carrying out the test. A full time technician, Mr. Lucias A. Davis, has greatly facilitated our investigation by his faithfulness, interest, and full knowledge of the technic as developed.

A few investigators have repeated our work. H. Hirsch (223) in his study of cyclical bloods has corroborated our findings in every detail. Margaret Smith (419) in a study of pregnancy bloods, using a very slight variation of our method, has likewise confirmed our results. The work of E. Fels (120) fully confirms our work although he too has used a variation in method which is comparatively insensitive. In addition, from personal communications, those workers in laboratories here and abroad, who have begun to take up the method, are obtaining results corresponding largely to our own, but as yet unpublished.

J. C. Janney (505) since the completion of my manuscript, has published a preliminary report based on 203 blood readings. The number of cases from which this material was derived was not stated. The author finds considerable differences in results from those reported by us. The main differences consist in the fact that he obtains many negative readings premenstrually and fewer positive readings in pregnancy.

> In studying the article of Janney, it is apparent that he has altered our technic in a number of fairly essential ways. He keeps specimens in ether in the ice box for varying periods of time. This, as I have warned in my description of the technic, allows the peroxide present in the ether, to affect the hormone unfavorably. The fact that Janney uses 50 grams of sodium sulphate in drying the 40 c.c. of blood is also of importance. In other words, he uses 20 grams more or a 66 per cent greater amount of sodium sulphate. The ether extract therefore will not be as perfect. And finally he centrifuges the ether extract. As is well known, the hormone is readily carried down by means of surface adsorption on any precipitate.
>
> Furthermore, from Janney's article, the type of patients used for the test cannot be judged at all. In our own work the normals have been based on perfectly healthy, cyclical women. A second group consisted of less normal individuals who still, however, were cyclical, showing either slight or no premenstrual rise as noted on page 188 and finally the sterility group consisting almost entirely of women with a low premenstrual threshold.
>
> More detailed analysis of Janney's results than is possible from the data he presents, may therefore conform very closely to our own. From his pregnancy results I am however inclined to conclude that the modification in method which he has adopted is not as good as our own original technic.

At the very beginning of our studies, even before Laqueur (264), Coward and Burn (84) and more recently Kahnt and Doisy (247a) emphasized the varying susceptibility of animals and drew attention to the many possibilities of error, we were fully aware that we were forced to employ a qualitative method to obtain quantitative results. It was at first difficult to determine the minimum amount of blood necessary to obtain an ample mouse unit, leaving sufficient margin for variations in susceptibility of the individual castrated mice used for the test. After many preliminary tests, we first settled on the quantity of 35 c.c. but eventually increased this to 40 c.c. which has remained the amount we have employed for the last 3 years. It follows from the fact that as much as 40 c.c. are needed for each test that we have been unable to run duplicate tests as a routine and that only in very rare instances have we been able to run more than a single test at one time from the same patient. This has its distinct disadvantages as, occasionally, in spite of the greatest care, the injected mouse may die before a final reading can be obtained and thus disturb or interrupt continuous series of blood examinations, or delay a report at a crucial time.

Our aim throughout has been to shorten the time required for the test as much as possible. We have devised a technic (159) by means of which less than two hours are required between the time of abstracting the blood and injecting it into the test mouse. Then, however, 48 hours must elapse before a final reading can be obtained. Our further efforts have been directed toward simplifying the manipulations and materials required, so as to make them available for those who possess only the simplest laboratory facilities. It has also been our aim to minimize the loss of active substance during the preparation of the extract to be injected, because no method has as yet been devised by means of which all loss of hormone can be avoided during concentration. It should, of course, be self-evident that we have tried such methods as injection of the untreated blood serum as proposed by Binz, Trivino, and Fels, but we have found that even in pregnancy this method does not give clear-cut results.

Development of Method

In our first paper (154) large quantities, 300 c.c. of estrous blood, were extracted by means of alcohol and benzene. This left as much as 75 mg. of crude extract which was injected into castrated rats.

In order to increase the delicacy of the method and reduce the amount of blood required, a smaller test animal, namely the castrated mouse, was chosen. In 1926, the results obtained were first described by Frank and Goldberger (155). The technic at that time (Frank and Goldberger, 1926, 157) consisted in using 35 c.c. of vein blood which was run into 95 per cent ethyl alcohol, the alcohol being driven off and the sediment extracted with ether. The ether residue was emulsified in a small quantity of water and injected into the test mouse. It was found that by this method much apparently toxic material reached the test animal and caused a high mortality. For this reason we developed the present simplification and improvement of technic which up to the present we have been unable to better.

Present Technic

In 1928, we (Frank and Goldberger, 159) published the following technic which we still employ. It is extremely important to cultivate the utmost uniformity and to practice the minutest care in execution of every step as indicated. Only by these means is it possible to avoid a great percentage of error. For any one beginning this work, it is necessary to devote a period of at least two months to the study and practice of the technic before placing any reliance upon the results. We have instructed a number of physicians whose laboratory training has been rather limited, in our technic, and from their reports we conclude that they have had no trouble in perfecting themselves in the use of the test. Usually two entire days spent in our laboratory, devoted to watching every step, have sufficed to prepare them for independent work.

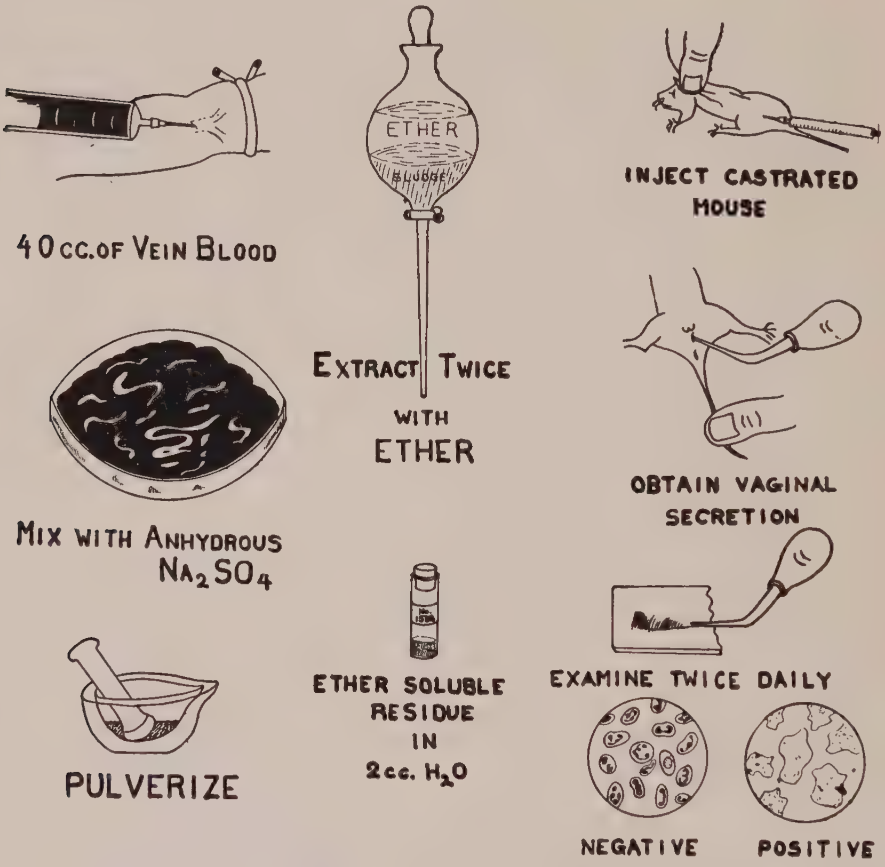


Fig. 45. Graphic Schema of the Female Sex Hormone Blood Test.

The details of the technic are as follows:

1. 40 c.c. of vein blood are removed, usually from an arm vein by means of a 50 c.c. glass syringe.

2. The blood is at once distributed in a large (6 inches in diameter) petri dish, the bottom of which has just previously been evenly covered with 30 grams of finely powdered anhydrous sodium sulphate (Na_2SO_4). The sodium sulphate before use is kept in an air-tight jar. The blood and the powder are rapidly and intimately mixed by means of a metal spatula so as to form a thick paste. During the first 10 minutes of the drying process, the mixture is continually stirred with the spatula in order to avoid the formation of large hard lumps. As the drying proceeds, the solidifying layer at the bottom of the dish is cut into numerous squares by means of the spatula in order that its removal is more easily effected at the time complete drying has taken place. This requires at most one-half hour.

3. The small, dry, brittle, hard lumps are finely triturated in a mortar.

4. The resultant smooth, fine, red-brown powder is poured into a separating funnel and shaken for 10 minutes with 100 c.c. of ether (we use anesthetic ether of a make which contains a minimum amount of peroxides). After settling for 10 minutes, the supernatant ether is poured off and preserved. To the residual sludge in the separating funnel an additional 75 c.c. of ether is added and again shaken for 10 minutes. After settling for 10 minutes the supernatant ether is again poured off and combined with the previously obtained ethereal extract.

5. The combined ether extracts are placed in a bowl shaped glass evaporating dish in front of an electric fan and the ether driven off completely and rapidly if time is an important factor. If for one reason or another greater haste is of importance, the ether may be driven off by placing the evaporating dish on a water bath under a hood, the entire time of drying then requiring only 20 minutes.

6. The residual sediment is rubbed up under sterile precautions with 2 c.c. of sterile water so as to form an emulsion and is then ready for injection.

7. The watery emulsion is injected in 3 equal portions at 3-4 hour intervals into a previously castrated adult white mouse (the castration performed at least 14 days previously and the spreads of the mouse remaining continuously negative). Long needles, 1 1/2 inches long, 24 gage, and tuberculin syringes are employed. The needle is carried from near the tail end of the mouse, subcutaneously to near the shoulder region in order that leakage from the needle tract is reduced to a minimum. After injecting the animal, 1/4 c.c. of sterile water is sucked up through the needle of the syringe in order not to lose the small amount of active substance remaining in the syringe. This residuum is added to the stock vial containing the remaining blood emulsion to be injected and this precaution is taken with the next succeeding injection, the final injection including all the residuum in the vial.

8. A vaginal spread is made 24 hours after the last injection has been given. On the following morning and afternoon other spreads are made,

the final reading being made 48 hours after the last injection. As a matter of precaution, in order not to overlook delayed reactions or the persistence of reaction (which signifies an extra strong reaction) a final late spread is made the following morning.

To summarize the procedure; it consists in the abstracting of blood, drying by means of anhydrous sodium sulphate, full ether extraction, emulsification of the ether residue in water and injection in the test mouse. For details, accuracy, and other facts concerning the Allen and Doisy vaginal smear reaction, see page 88.

Avoidance of Errors

The commonest cause for errors are due to allowing the blood to remain too long in ether, where the presence of peroxides may oxydize and inactivate the hormone. If the ether residue is kept too long in water, deterioration may also take place. We therefore prefer driving off the ether slowly by spontaneous evaporation during the night and completing this process in a few minutes by means of the electric fan in the morning. The residue is then at once emulsified in water and the injections begun without delay (8:45–11:30 A.M.–3:30 P.M.). It is hardly necessary to emphasize that every one of the steps must be taken according to the usual quantitative technic, and that every step must be performed in an identical fashion if comparable results are to be looked for.

Experience and skill will reduce the leakage from the needle tract in the test mouse to a very rare occurrence. If such leakage takes place it must be noted, in order that a negative result in certain instances is accounted for, or that if the leakage has been great, that the given test can be discarded.

The reading of the vaginal smears is of such extreme importance that special emphasis must be placed upon it and I shall enter into considerable details in pointing out the avoidance of errors and pitfalls.

Mice to be Employed

In our laboratory we are in the habit of keeping on hand a minimum of 40 to 50 castrated mice ready for injection. These mice are adult white mice of a uniform strain. They are castrated through an abdominal incision, great care being exercised in removing not only the ovaries but the adjacent peritoneum and Fallopian tube. Since this liberal excision has been practiced, reformation of ovaries with consequent spontaneous ovulation, has become the utmost rarity (less than 1 in 200).

It is essential for beginners to control the castrated mice for a period of at least 4 weeks by means of daily spreads in order to make sure that no cycle is present, as a spontaneous positive reaction would entirely obscure and nullify the test, besides proving misleading.

It is well the evening before a series of tests are to be undertaken, to select the mice to be used for injection. From the mice to be utilized, vaginal spreads are taken which on examination must be definitely negative (0). We have found it convenient to mark mice for identification by means of distinguishing colors, as follows:

carbol fuchsin = 1–10
methyl violet = 10–20
picric acid = 20–30
orange = 30–40
purple = 40–50
green = 50–60
red and yellow = 60–70
purple and orange = 70–80
yellow and blue = 80–90
blue and red = 90–100

In addition each mouse in the series is provided with ear marks which further identifies it. See Fig. 46.

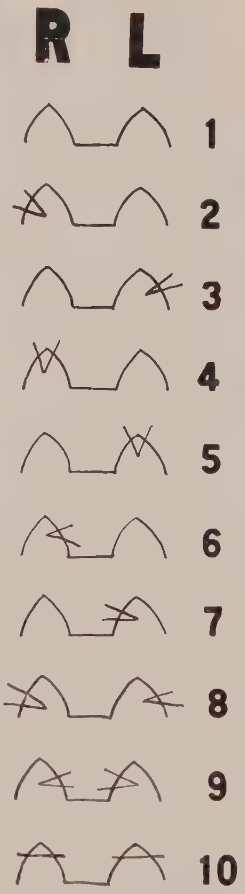


Fig. 46. Method of Marking Mice.

Right and left ear of the mouse facing the observer is depicted. The triangles represent small segments of the ears snipped out with sharp scissors. This method of marking is permanent. R = Right. L = Left.

Food of Mice

The mice are provided daily with stale white bread, dried corn on the cob and fresh milk. Once each week a few drops of codliver oil are added to the milk. Once each week they receive a few leaves of fresh cabbage. Under this regime the mice and also rats thrive and propagate freely. Ten mice are kept in each cage. Figs. 47–48.

Care and Cleaning

Each morning the inhabitants of each cage are "poured" into a large glass jar while their cage is cleaned. The bottom is removed, emptied and scalded. A fresh piece of newspaper covers the bottom. A small handful of non-absorbent cotton is supplied for shelter and warmth. Then food is added and the mice replaced. Each cage is furnished with an empty pasteboard box (in which ether cans are supplied). In this the mice like to hide and seek shelter.

Recording of Experiments

The following simple record system has proved sufficient.

NO.	SOURCE	NO. OF MOUSE	TOTAL AMOUNT INJECTED	INJECTIONS			READINGS					FOLLOW UP
				8:45	11:30	3:30	1/15	1/16	1/17	1/17	1/18	
				1/15/1929					A.M.	P.M.		
1052	Blood	16	2 c.c.	0.8	0.8	0.4	0	0	2	3	2	
1053	Cyst fluid	18	3 c.c.	1	1	1	0	0	2+	3	0	
1055	Urine Pregnancy	19	1.5 c.c.	0.5	0.5	0.5	0	2	2+	4	2	

In order to avoid errors, we have devised very simple arrangement for injecting large numbers of bloods almost automatically, as the following photograph shows, the vials being arranged in series and a corresponding tuberculin syringe being placed next to each vial. See Fig. 49.

The Taking of the Spreads

Twenty-four hours after completion of the injections, spreads are taken from the test mice by the following technic which has proved satisfactory in our hands.

A large number of small glass pipettes are prepared from glass tubing and kept on hand, a single pipette being used once only and then discarded until cleaned. The technician takes up the test mouse and holds her belly upward. A marked clean glass slide is held in the left hand while, by means of a bulb attached to the pipette, two drops of water are drawn up and injected into the vagina of the mouse. By slightly compressing and relaxing the bulb, this water is intimately mixed with the vaginal content and then withdrawn in the pipette. Then the at most two to three drops of turbid fluid are ejected on the slide and spread with the heel of the pipette. The slide is air-dried, fixed in the flame, and instantaneously stained by a 1 per cent solution of thionin which is at once washed off with running water.

Numerous fields of the spread are examined with a low power of the microscope and a decision as to the classification of the spread is arrived at. Clean, clear-cut spreads are readily evaluated, but experience is required properly to classify spreads which are uneven in different fields or in which gradual transitions from one character to another are noted. The following classification has been adopted by us throughout our work.

0 = untreated castrate; leucocytes, mucus, a few nucleated epithelial cells.
1 = negative; predominance of leucocytes; increase in nucleated epithelial cells.
2 = still negative; presence of numerous leucocytes; predominance of nucleated epithelial cells.
2+ = weak positive; occasional leucocytes; nucleated epithelial cells.
3 = threshold reaction; no leucocytes; nucleated epithelial cells.
4 = strong reaction; epithelial non-nucleated scales exclusively.
See Fig. 50.

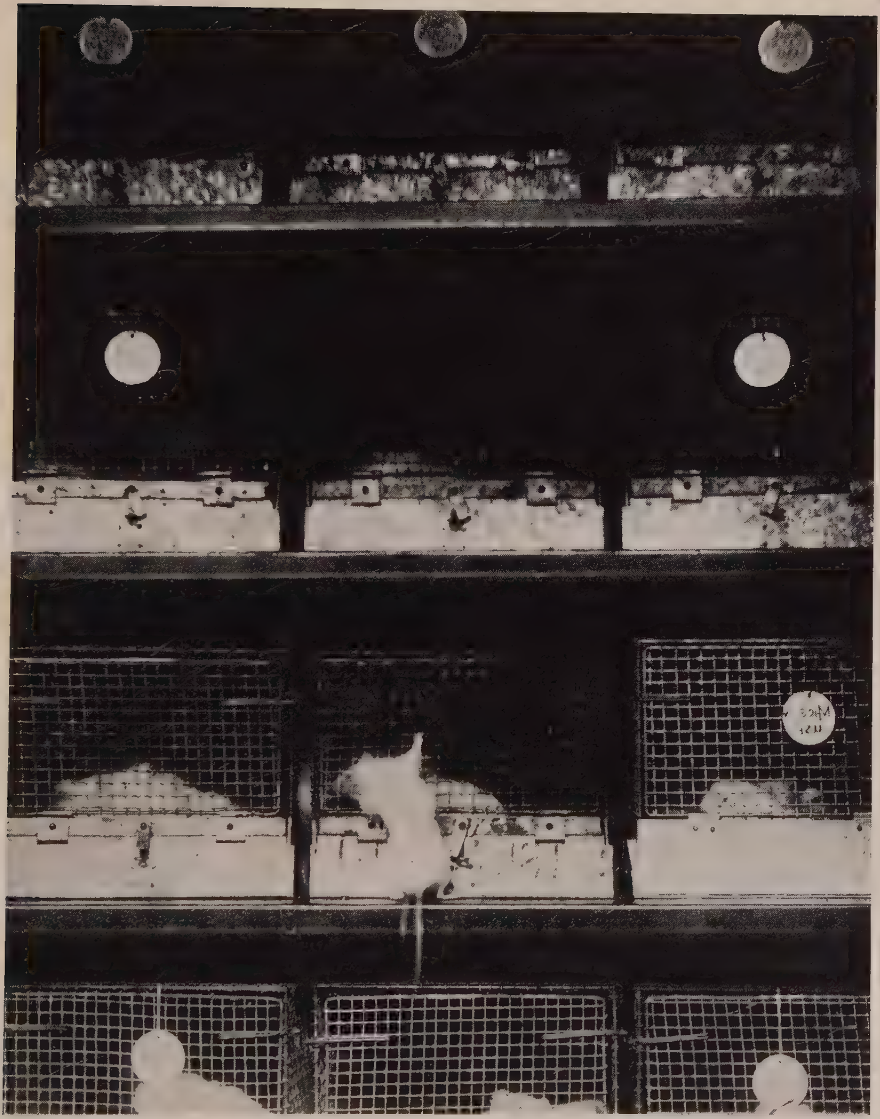


FIG. 47. NEST OF CAGES AS CONTAINED IN METAL STAND.

Reading of Results

Although a 2 really signifies some slight reaction, we have found it necessary to consider 2+ the lowest limit safely assignable to a weak positive reaction. On the other hand 3, signifies a good threshold reaction and 4 is a strong reaction, usually characteristic of pregnancy or of an undue cyclical accumulation of blood in the non-pregnant.

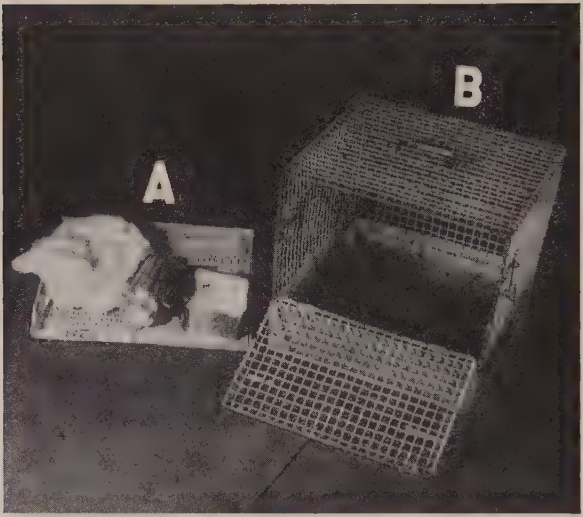


Fig. 48. Empty Cage.

A. Bottom of cage covered with paper containing non-absorbant cotton, dried corn on the cob, stale bread. B. Top of cage with hinged door open.

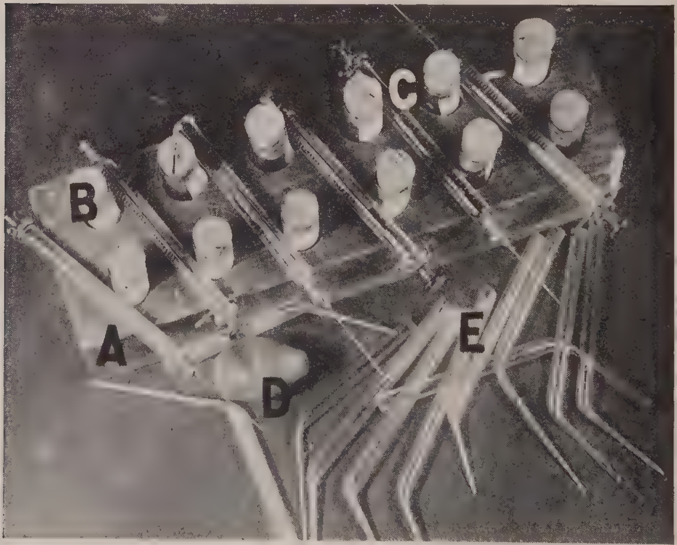


Fig. 49.

A. Stand for vials. B. Vial containing aqueous emulsion of blood. C. Tuberculin syringe. D. Rubber bulb. E. Numerous glass pipettes used for taking vaginal spreads.

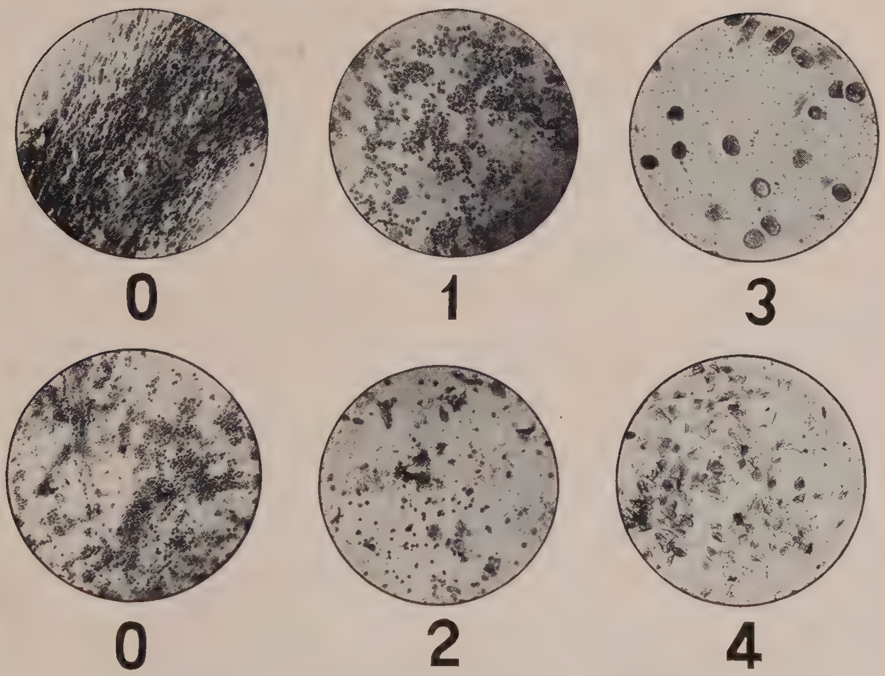


FIG. 50A. LOW POWER PHOTOMICROGRAPHS OF VAGINAL SPREADS FROM MOUSE.

The upper O represents a negative spread containing leucocytes and mucus. The lower O contains merely leucocytes. (1) Negative—leucocytes with a number of epithelial cells. (2) Negative—numerous nucleated epithelial cells with occasional leucocytes. (3) Threshold reaction—nucleated epithelial cells. (4) Strong reaction—squamous epithelial cells.

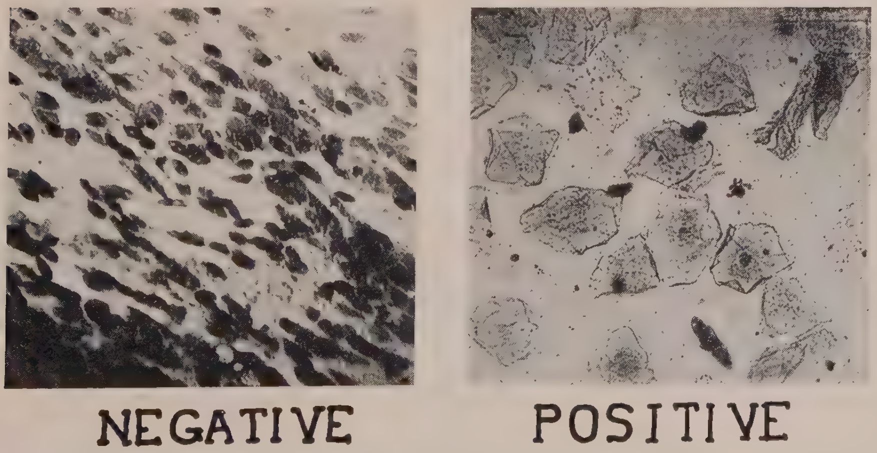


FIG. 50B. HIGH POWER PHOTOMICROGRAPH SHOWING NEGATIVE (0) AND POSITIVE (4) SPREADS.

Examination of Menstrual Bloods

It has been our experience that of early menstrual blood, that is, blood obtained during the beginning of the first day of the flow, 5 c.c. are sufficient to give a positive reaction in the normal case. As menstrual blood appears to be toxic and also contains many bacteria, we find it necessary partly to sterilize and prevent further decomposition, by putting it with three times the amount of ethyl alcohol, in other words, to run it through using our old method, p. 179. The ethyl alcohol is removed by evapora-

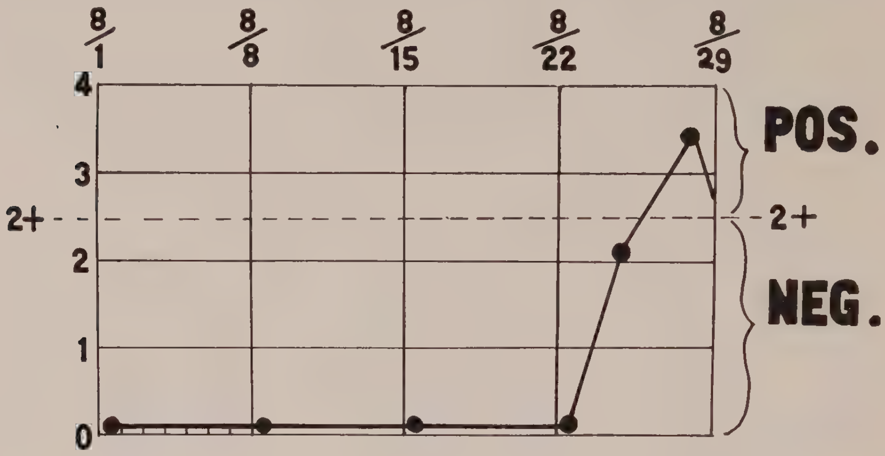


FIG. 51. TYPICAL GRAPH SHOWING RECORD OF POSITIVE EXPERIMENT.

This type of graph will be used throughout without marginal numbers. The dates will appear on the top line. The strength of the reaction can be readily gaged by counting lines from the bottom upward, the base line being 0, the top line representing a 4 reaction.

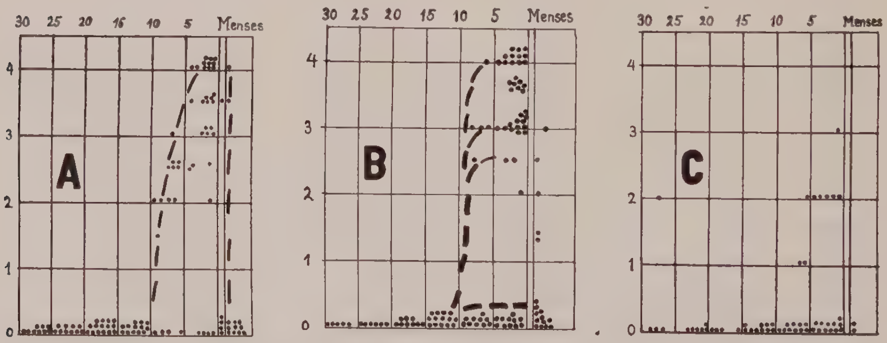


FIG. 52. GRAPHS SHOWING THE REACTION AS DETERMINED FROM THE VENOUS BLOOD.

A. In strictly normal women. B. In cyclical women many of whom had gynecological diseases. C. In sterile women.

tion on the water bath, the residuum extracted twice with ether, and the ether residue, resulting after evaporation of the ether, emulsified in 2 c.c. of water for injection.

STATISTICS

The following summary will show the number of tests performed and the type of material. The basic investigation naturally centered on norms, which included cyclical patients, pregnancy patients, and menstrual blood tests. In addition a large number of urines were examined.

Summary of 1262 Tests

Material	No. of Patients	No. of Bloods Tested
Normal Cycle	97	172
Pregnancy	159	202
Pathological bleeding	72	130
Amenorrhea	55	215
Sterility	17	53
Absent Vagina (Sex)	8	40
For diagnosis	36	52
Miscellaneous	38	75
Male Individuals	56	71
	Total 538	Total 1010

Urines Tested	
Males	30
Pregnancy	19
Cyclical	127
Miscellaneous	38
	214

Human Tissues Tested	
Placenta	11
Cord Blood	2
Amniotic fluid	2
Cyst fluid	7
Corpora lutea	3
Embryo	1
Bile	10
Saliva	2
	38

Not included in the above are *48 cases* with *82 bloods discarded* for lack of history, patient lost sight of, test mouse died, etc.

THE BLOOD IN NORMAL WOMEN

In the regular, fertile, and normal woman with no serious pathological conditions, examination of 108 bloods from 66 patients, showed that in the great majority 40 c.c. of blood did not contain a mouse unit until about the 7th day before the onset of the next menses. An abrupt rise then takes place, 40 c.c. of blood then containing either enough to produce a 3 or 4 in the test mouse. In a small number of cases the reaction is less strong, being between 2 and 2+. In a very small percentage of cases no premenstrual rise is noted during this period. Immediately after the onset of the menses, an abrupt drop regularly takes place. The normal cyclical condition is shown on the accompanying graph. Fig. 52A.

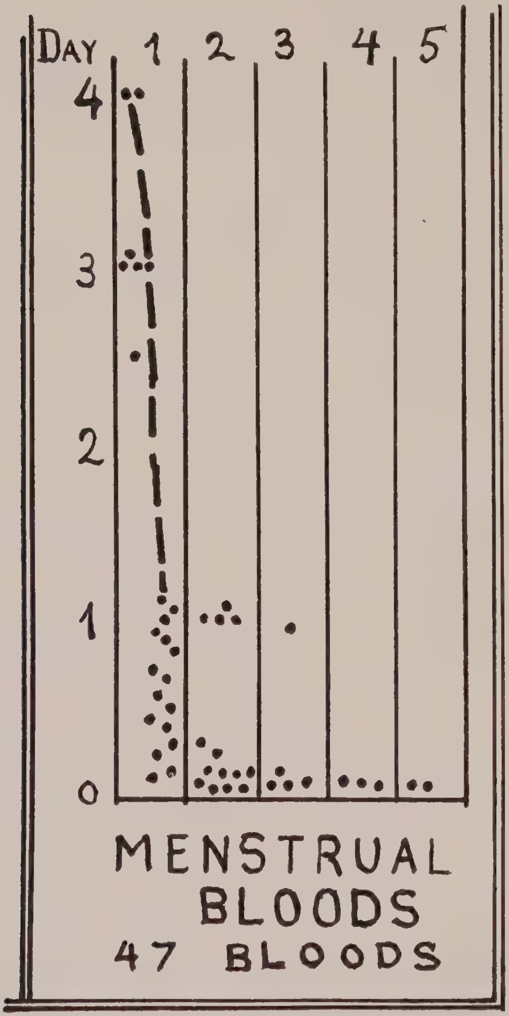


Fig. 53. Graph of Menstrual Bloods.

The early menstrual blood contains large amounts of female sex hormone. After the first day this rapidly drops to 0.

The first menstrual blood, on the other hand, contains a large amount of female sex hormone in concentrated form. Four and 5 c.c. of blood, will contain a full M.U. if obtained on the first day of the menses. Thereafter a rapid drop to 0 is again noted (see the graph). Fig. 53.

If less strict criteria are used in selecting cyclical women and the general run of women seen in a gynecological clinic are examined, it will be noted that the vein blood of a comparatively larger group never reaches the +3 level, and that the number who show no premenstrual rise, although their menstruation is normal as to time of occurrence and amount, is greatly increased. Fig. 52B.

The variations are well illustrated by the 3 charts shown, in which tests performed on 3 different groups of women have been collected and graphically depicted.

The same increase in negatives is likewise noted if the menstrual blood of menorrhagia and other pathological cases is examined. Under these conditions the early menstrual blood may be negative and menstrual blood obtained as late as the 4th or 6th day may contain a considerable amount of hormone.

A normal cycle in the urine had been found as early as 1925 by Loewe (311). It is extremely difficult and tedious to carry out this investigation. Our own studies differ somewhat from Loewe's in that we find two peaks in the tests, the one approximately extending from the 17th to the 24th day *after* the beginning of the last period and another one often showing during the first few days of the menstrual flow. In certain cases, with low premenstrual blood level there appears to be a permanent, high excretion in the urine, in other words, a low kidney threshold for the hormone. The theoretical importance of this will be taken up in the next paragraphs.

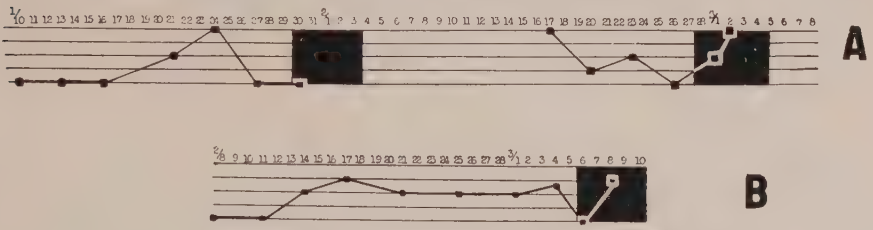


FIG. 54. GRAPHS OF 2 URINES. A—No. 1698—normal female. B—1392—sterile patient.

> We have found it necessary to employ 200 c.c. of urine for the test, which we habitually run in duplicate. 200 c.c. of fresh urine are shaken for 10 minutes in a separating funnel with 100 c.c. of ether. The ethereal fraction, after 10 minutes of settling is poured off and kept. The urine is again shaken with 50 additional c.c. of ether.
>
> The ethereal fractions are united. The ether is evaporated, the residue taken up in 2 c.c. of sterile water and injected into the test mouse.

As yet we have been unable, in spite of long continued effort, to substitute the urine as a source for the hormone test in place of the blood.

The Mechanism Which Governs the Hormone Level in the Blood

In Part I, I have attempted to show, as is now generally accepted, that the primary activation of the ovaries takes place through the effect of the anterior lobe pituitary action. It would also appear that the normal cycle is largely due to the periodic elaboration of the female sex hormone resulting from the growth of the follicle and after follicular rupture, to the continued secretion of the active substance by the corpus luteum. If pregnancy supervenes, the corpus luteum persists and its action is in turn taken up by the placenta, for which reason we have spoken of the gestational gland. (p. 25).

In addition, the work of Parkes (359, 360) has made it likely that there is an emergency substituting apparatus by means of which any of the cells derived from the germinal epithelium can likewise secrete female sex hormone. Then, too, the observations of Aschner (27), Schröder (409), and Geist (176) have shown that protracted excitation of the tubular tract may be produced by continuous and exaggerated follicle growth.

The possible mechanisms to be considered then, are (1) an intermittent production of female sex hormone resulting from regular follicle and corpus luteum production; (2) inhibition of follicle and corpus luteum growth, resulting from inhibitory corpus luteum action (Loeb, 298; Papanicolaou, 354; Hisaw,225*a*; Parkes, 370); (3) a threshold excretion, the possibility of which is made likely by the discovery that female sex ormoneh is excreted in the urine and feces, and perhaps by means of other channels.

Threshold Level

Our investigations have shown that normally in the week preceding menstruation (the pregravid period) the blood level is 1 M.U. to 40 c.c. As we shall soon show, during pregnancy this level may reach the height of 1 M.U. for every 30-40 c.c. of blood. That there is an actual mechanism which governs the excretion of the female sex hormone is definitely shown by the fact that in spite of the tremendous overproduction of the hormone during pregnancy, the blood level remains constant.

Therefore, it seems justified to postulate, although absolutely convincing experimental proof is still lacking, that a threshold mechanism exists acting mainly through the kidneys and intestine. In the non-pregnant state from 2–5 M.U.L. (mouse units per liter) of urine are excreted daily, while in the pregnant state the excretion may reach as high as 10,000 M.U.L., Zondek and Aschheim (482). The excretion through the feces has been less carefully studied, the sole reports emanating from Dohrn and Faure (96) who obtained as much as 30,000 M.U.K. (mouse units per kilogram) from dry feces.

Excretion of the hormone through the menstrual blood now appears to us to be of minor significance, although the menstrual blood contains the hormone in 5 to 6 times the concentration in which it is found in the circulating blood. How small a rôle the menstrual excretion really plays is shown by those cases to which we shall again refer on page 256, namely, patients after hysterectomy, in whom the ovaries were left *in situ*, and in whom a regular blood cycle has been determined by us.

The Menstrual Mechanism

Histology of the Endometrium during Menstruation

According to Gebhard in 1899 (174) the uterine endometrium becomes pervious and the blood escapes largely by diapedesis, leakage through the blood vessel walls and through minute ruptures of the surface epithelium. Schröder (407) has described a different type of menstruation in which the functional layers (compacta and spongeosa) are exfoliated, leaving a raw wound, the base of which is formed by the unchanged basilar layer (for details see chapter on anatomy, page 36). In addition Corner (495) has recorded regular menstruation in macacus rhesus through an unchanged endometrium, which does not even show the pregravid (premenstrual) alteration, but resembles the picture we are accustomed to associate with resting endometrium.

Induction of Menstruation in Castrates

Zondek (481) has produced menstruation in a human castrate by repeated injection of female sex hormone (folliculin), followed by its abrupt withdrawal. The same phenomenon has been equally strikingly produced by Pratt and Allen (378) in castrated monkeys. These two sets of experiments show unequivocally that in its simplest form menstruation can be produced by loading of the endometrium by means of the female sex hormone followed by abrupt withdrawal of this activating agent.

"Loading" of the Endometrium

Further light is thrown upon this "loading" process by the following experiments performed by us. We (Frank and Goldberger, 159) have shown that the menstrual blood may contain female sex hormone in 5 to 6 times the concentration in which it is found the circulating blood of the same individual. If now by means of scarification and a cervical suction cup, blood is obtained from the portio vaginalis, this blood contains no greater amount of female sex hormone than is found in the blood of the general circulation. At this time, however, the menstrual blood already contains the hormone in 5 to 6 times that concentration. This therefore offers proof that the hormone is either stored in the endometrium or that as the capillaries traverse the endometrium, the hormone is selectively removed from the circulation, concentrated, and excreted with the menstrual blood into the uterine cavity.

The Cause of Menstruation

No matter whether the exfoliative condition described by Schröder (407), the transudation process discovered by Gebhard (174) or the unchanged endometrium noted by Corner (495) exists, bleeding does not seem to occur until the endometrium has been loaded or primed, and the stimulus then discontinued abruptly. Whether pathological bleeding, not due to mechanical causes, develops upon the same basis, I am quite unable to state at present.

Inhibition of Menstruation

Inhibition of menstruation under normal conditions has been explained upon a different basis by different investigators. Loeb (298) explains it by inhibition of follicle ripening due to cyclical growth of the corpus luteum with transitory inhibiting power. Under these conditions the mucous membrane is in a negative phase. In pregnancy the persistence of the corpus lutem may inhibit follicle ripening but the mucous membrane is in an advanced state of stimulation due to the high and continued level of female sex hormone in the circulation. During lactation, on the other hand, although according to Parkes (360–361) the inhibition is due to the corpus luteum of lactation, restraint of follicle ripening produces a low hormone level. Hence the mucosa again is in a resting stage and the uterus often in an advanced state of atrophy.

Inhibition of estrus by persistent and continuous follicle ripening has been noted in the ferret by Robinson (392). In the human being such persistent follicle ripening, according to Schröder (409) and to Geist (176) has been associated on the other hand, with persistent uterine bleeding.

The absence of menstruation is accounted for by failure of preparation or loading of the endometrium, which theoretically (and as since proved by our investigations) may result from one of two causes, *under-production* of female sex hormone by under-acting ovaries, or *over-excretion* of the hormone through renal and intestinal channels, in spite of normal or even hyper-active ovaries, if these thresholds are pathologically low.

Pregnancy Norms

As early as May 21, 1926, at a Meeting of the American Gynecological Society, we (Frank and Goldberger, 147) reported our first observations on the female sex hormone content of the blood in pregnant women. From the 8th week of pregnancy on, we regularly found a strong reaction in 40 c.c. of blood and not infrequently found a full M.U. in as little as 20, 25, and 30 c.c. of blood. This high hormone level was noted undiminished

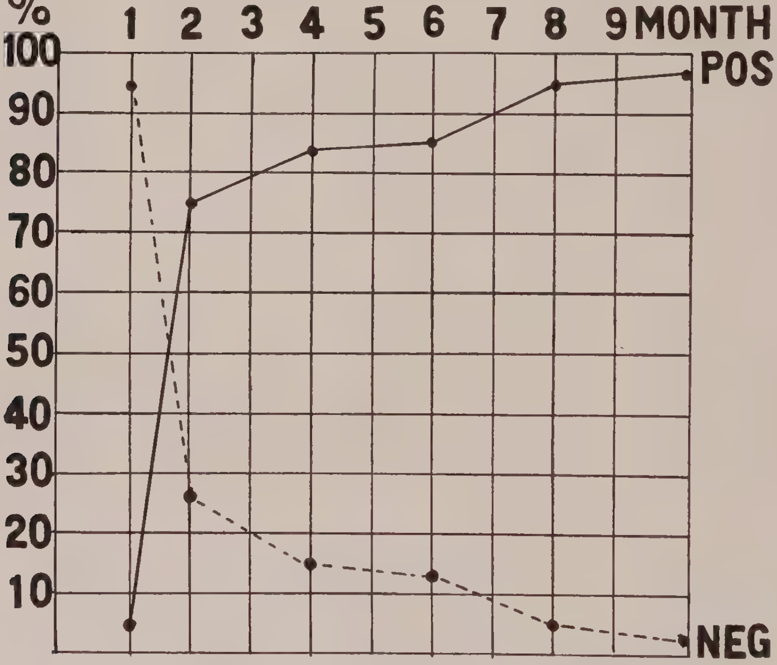
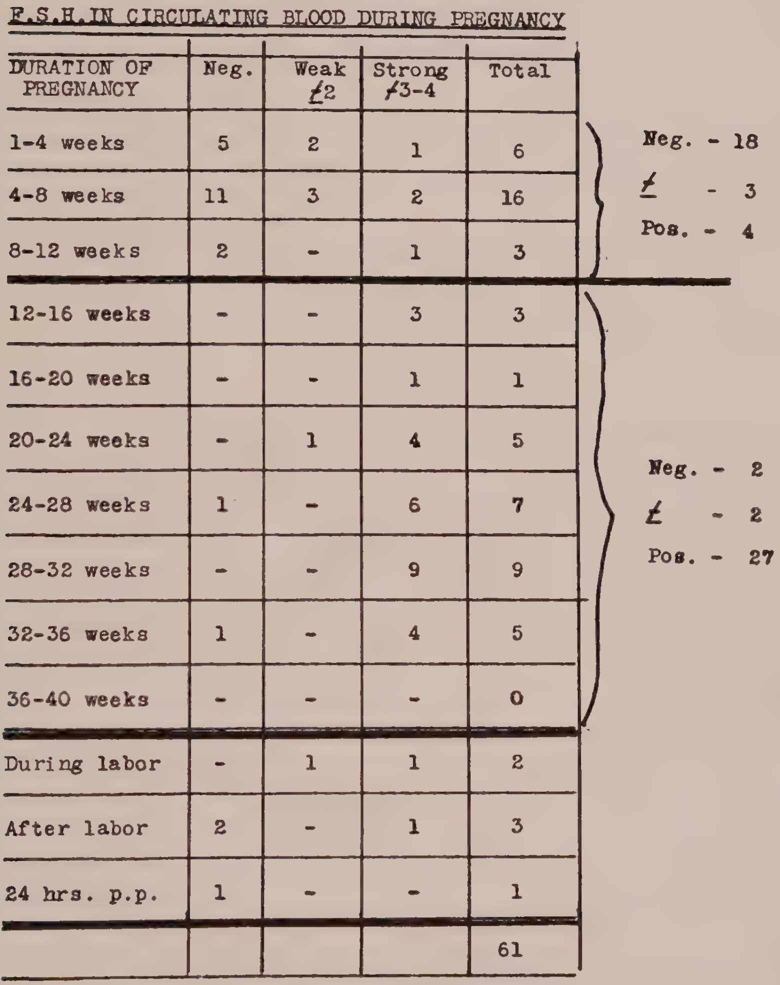


Fig. 55. Chart Showing Results of Blood Test in Pregnancy.

Solid line shows percentage of positive results at various months. Broken line shows negative results in pregnancy at various months and may be regarded as an iandiction of the percentage of error.

throughout pregnancy and continued, as can be seen from our chart, until the onset of labor. After delivery and with the expulsion of the placenta, the hormone level abruptly dropped within 24 hours, reaching the level we associate with the resting stage in the cyclical woman. These observations have been fully confirmed by Margaret Smith (419) and Fels (120) as well as Zondek and Aschheim (482) and others. The accompanying table shows that a negative phase exists immediately after impregnation, persisting until approximately the 8th week of pregnancy. How to account for



F.S.H. IN CIRCULATING BLOOD DURING PREGNANCY

DURATION OF PREGNANCY	Neg.	Weak ±2	Strong +3-4	Total
1-4 weeks	5	2	1	6
4-8 weeks	11	3	2	16
8-12 weeks	2	-	1	3
12-16 weeks	-	-	3	3
16-20 weeks	-	-	1	1
20-24 weeks	-	1	4	5
24-28 weeks	1	-	6	7
28-32 weeks	-	-	9	9
32-36 weeks	1	-	4	5
36-40 weeks	-	-	-	0
During labor	-	1	1	2
After labor	2	-	1	3
24 hrs. p.p.	1	-	-	1
				61

Neg. - 18
± - 3
Pos. - 4

Neg. - 2
± - 2
Pos. - 27

FIG. 56. CHART SHOWING AMOUNT OF FEMALE SEX HORMONE IN THE CIRCULATING BLOOD DURING VARIOUS MONTHS OF PREGNANCY.

this period of low level is difficult to say. Possibly the corpus luteum of early pregnancy rapidly loses its producing power before the chorion assumes its full activity in the production of female sex hormone. Our experiments have shown that while 1 gram of placenta at term, by means of our rather crude extraction method, contains a full M.U., it may require as much as 4 grams of chorion of the first 4 to 6 weeks to give a M.U. On the other hand, it is necessary to bear in mind that the amount of active substance extracted from a given tissue is no index of the amount produced by that tissue.

That the placenta plays a predominant rôle in the production of the female sex hormone is shown by the fact that if the placenta is retained after expulsion of the fetus, and this applies as well to early pregnancy as to term conditions, the high pregnancy hormone level continues until the intimate functional connection between uterine wall and placenta has been interrupted. This was strikingly shown, as if in a laboratory experiment, by a patient in whom, after the delivery of a full term child, the placenta remained *in situ* for 17 days post partum, the blood level remaining correspondingly high until the placenta had been manually removed. See page 252.

Hormone Excretion in the Urine

Aschheim and Zondek (482) showed that a huge amount of female sex hormone is constantly excreted in the urine of pregnant women. In 1928 they claimed that in the 1st to the 8th week, 3 to 600 M.U.L. were excreted, in the 3rd to the 7th months, 5 to 7,000 M.U.L., in the 7th to the 10th month, 6 to 10,000 M.U.L. Veler and Doisy (453) confirmed the high hormone excretion in the urine and showed how rapidly it disappeared post partum as their following table shows.

Just before delivery

R.U.L.	480	500	630	710	625	830	1000	470	1240
Wgt. of 1 R.U. in mg.	–	0.06	0.06	0.12	–	0.06	0.14	0.22	0.06

Post Partum

Hours P.P.	8.5	37	48	72	96	144	192	264
R.U.L.	640	500	80	22	20	L5	L5	L4

Anterior Pituitary Lobe Excretion in the Urine of Pregnant Women

Aschheim and Zondek (24) also showed by means of the reaction produced on immature mice, that a large amount of anterior pituitary hormone is excreted in the urine of pregnant women immediately after conception. They recommended this as a test for pregnancy. See page 19. Our own experience with the test has been most disappointing, as on the one hand, we were unable to keep in stock the large number of immature mice needed for performing the test, and secondly, the immature mice regularly died during the course of the injections long before readings could be taken.

In the previous portion of the book we have already discussed the tests proposed by Binz (46), Trivino (443), Louria and Rosenzweig (512), Mazer and Hoffman (330), Fels (120), Smith (419) and others, for diagnosis of pregnancy. None of these tests are fully trustworthy. Our own test, (see page 247) applied to pregnancy, has numerous disadvantages, including the fact that until the 8th week no result can be expected and that a positive reaction is also regularly found if menstruation is impending. Nevertheless, in spite of these handicaps, the test has helped us in differential diagnosis in numerous instances, as will be shown later.

Female sex hormone in the bile is of great theoretical interest and is of importance in explaining the occurrence of female sex hormone reaction in the blood of males and in the urines of males. See page 115.

Female sex hormone in food. We have found as much as 1 M.U. of female sex hormone in 75 grams of boiled, mashed potatoes, showing that the average diet must contain a considerable and not negligible quantity of female sex hormone if this substance is stored in the human body.

Vaginal Spread and Excisions in the Human

With the exception of Papanicolaou (355) all investigators have failed in their efforts to utilize the vaginal smear in the human. Papanicolaou claims to be able to diagnose pregnancy and other conditions from the human smear. Adler (1) has studied excisions from the vaginal mucosa and has established the occurence of certain cyclical changes in the epithelial layer, which somewhat resemble the changes found in rodents, but to a much less striking degree. This method does not appear to lend itself to clinical investigation.

SUMMARY

Our investigation has enabled us to develop a technic by means of which the contents in female sex hormone of the circulating and menstrual blood can be measured. This technic likewise lends itself to the study of the hormone in the urine or other excretions. By means of this method we have been able to determine norms both for cyclical and pregnant women. Our further work concerns itself with the study of deviations from the normal.

CHAPTER IX

EXAMINATION OF THE FEMALE PATIENT

IN SPITE of the fact that this monograph deals mainly with the study of the female sex hormone, it would be illogical and one-sided not to stress the extreme importance which the general examination of every patient plays in evaluating the presence of abnormal conditions. Inexcusable errors in diagnosis and treatment are made because this factor is so often overlooked. The necessity for constantly keeping in mind the constitutional and general makeup of each patient accounts for the considerable detail with which I shall describe this examination.

What Constitutes a Normal Woman?

Definition of a normal woman. The normal woman begins life normally, shows normal development from infancy onward, and at the time of adolescence common to her race, surroundings, and climate, usually between the ages of 9 and 14, developes the signs of puberty.

At the time of puberty, her secondary sex characters are manifest. These include the feminine pelvis, female hair development, female fat distribution over the breasts, nates, and mons, the small female larynx, the gradual or abrupt arrest of growth, due to closure of the epiphyses, as well as the development of a female psychical outlook and heterosexual inclinations.

The adolescent develops a menstrual cycle which recurs every 26 to 32 days. The duration of the menstrual flow varies generally between 3 and 7 days. The amount of blood lost appears to be between 50 and 60 c.c. during a period, but may reach double or triple that amount without being considered abnormal. The menstrual bleeding is rarely exactly periodic, variations of 1 to 3 days being rather the rule than the exception, even in regularly menstruating females.

The normal woman suffers little pain, but most women feel minor disabilities due to the menstrual phenomena, such as irritability, lassitude, headaches, and backache at this time. These phenomena have to be recognized and taken into account by industrial corporations employing women, by girls' schools and colleges.

Premenstrually, a slight mucoid vaginal discharge is normal. Increase in size of the breasts and the presence of colostrum may be considered physiological. The fundus of the uterus increases at least 1/3 in size preliminary to the period, with some softening of the supravaginal portion of the cervix with consequent resemblance to Hegar's sign of pregnancy. Often the one or the other ovary is found enlarged due to the presence of the corpus luteum.* These symptoms disappear rapidly with the onset of the menstrual flow. Whether this sudden deturgescence corresponds to the unloading of the female sex hormone stored in the mucosa or extracted by the endometrium, or whether it is due to the excretion by other channels, must remain open for the moment.

The normal woman, when mated to a potent partner, conceives readily. The researches of Corner (74) Allen and Pratt (11), and others, show that ovulation takes place between the 12th and 14th day after the onset of the preceding menstruation. This therefore should be the optimum time for conception, but in the human female no time in the cycle has proved a "safe" period. Little light has been thrown upon this phase of the subject by means of attempts at artificial insemination (Rohleder, 521).

After conception has taken place, a period of gestation, lasting in the human female approximately 278 days, ensues. The extra demands made upon the genital tract and the entire body by the parasitic ovum appear to be readily met, largely through the stimulating agency of the female sex hormone. The large amount of female sex hormone continuously secreted during pregnancy is amazing. That large amounts may be needed to produce the uterine, vaginal, and mammary hyperplasia incident to normal gestation and labor is readily understandable. The overproduction reaches such extravagant heights that literally thousands of M. U. of female sex hormone are daily excreted through the urine and feces, showing a great prodigality on the part of nature.

> The special sensitizing effect of the corpus luteum on the uterine mucosa needed to enable the fertilized ovum to embed is again referred to in passing. The most recent experiments of Allen and Corner (12a) in which pregnancy in the rabbit continued after very early ablation of the ovaries, if corpus luteum extracts were given, sufficiently proves this.

Gestation culminates in labor, which, depending on the normalcy of the individual, results in more or less damage to the birth canal. Twenty-four hours post partum the blood hormone level is again down to that of the resting period. In consequence of this reduction the puerperal involution of the uterus takes place rapidly and is completed within 6 weeks. The stimulated and hyperplastic breasts on the other hand, now develop secretory activity which shows itself in the production of milk. Retention of the placenta post partum, if functional contact with the uterine wall persists, inhibits milk secretion, which fails to occur until at least three days after the placenta has been removed.

The menstrual cycle may or may not return during nursing. In a large number of women after 3 or 4 months have elapsed, menstruation is re-established even if lactation is continued.

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* Emil Ries (520) of Chicago has described alternating transitory ovarian enlargement due to this cause.

After completion of lactation the normal cyclical changes are resumed until again interrupted by another pregnancy. After the age of 42-50 years has been reached, a gradual or abrupt cessation of ovarian activity produces the menopause. This, in brief, is the sexual life history of a normal woman. Beyond a certain latitude, deviations from this model are pathological. A full description of all phases may be obtained in Schröder's encyclopedic book on menstruation (409).

History Taking

The following points are of special importance in the present connection.

Family history. Number of brothers and sisters, fertility of mother and sisters, presence of congenital anomalies in other members of the family, consanguinity, hereditary diseases.

Previous history. Time at which patient began to walk, onset of puberty, changes in regularity of menstruation, amount or character of menstruation, presence or absence of dysmenorrhea, previous operations or treatment, previous ailments, photographs of patient at various periods of life, in cases of sterility duration of contraceptive measures, report of husband's semen; history of exposure to lead poisoning or to X-ray.

Present history. Changes in menstruation, last menstruation, etc.

Frequently from the history, the time of onset of a given disturbance may be determined. Onset in childhood (Frank, 151) often proves an hereditary basis. In adolescence the cause may be ascribable to remediable defects in hygiene, while the onset at maturity often results from the stress of sex life or the undue demands made for the struggle for subsistence. All of these differ materially from diseases due to the involution of senescence.

General Examination

General examination is of the utmost importance. Too often examination is limited to a given sphere, depending upon the predilections and training of the examiner. The general characteristics of the patient include the following—

Stature—short, normal, long.
Nutrition—thin, normal, obese.
Weight—under, normal, over.
Strength—asthenic, normal, strong.
Response—slothful and stolid, normal, alert and quick.
Skin—soft, normal, coarse.
Hair—hypotrichosis, normal, hirsute.
Blood pressure—low, normal, high.
Pulse rate—slow, normal, rapid.
Coloring—pale, normal, florid.
Teeth—spacing, size, shape.

Fingers—short, normal, long.
Pelvis—neuter, normal, male.
Fat distribution—neuter, normal, male.
Voice—high, normal, bass.
Psychic state—neuter, normal, oversexed, homosexual.

Many factors will be automatically observed by the experienced examiner. Instability of the autonomic system will show itself by flushes, transient erythematous eruptions, sudden general, or local sweats, exaggeration of tremors. Protrusion of the eyeballs (exophthalmos) and associated lid symptoms (Stellwag's sign, etc.) (Fig. 57A) as well as the size of the thyroid gland are deceptive (see Fig. 57B). A thyroid gland may be enlarged but underfunctioning, and normal in size yet overproducing thyroxin.

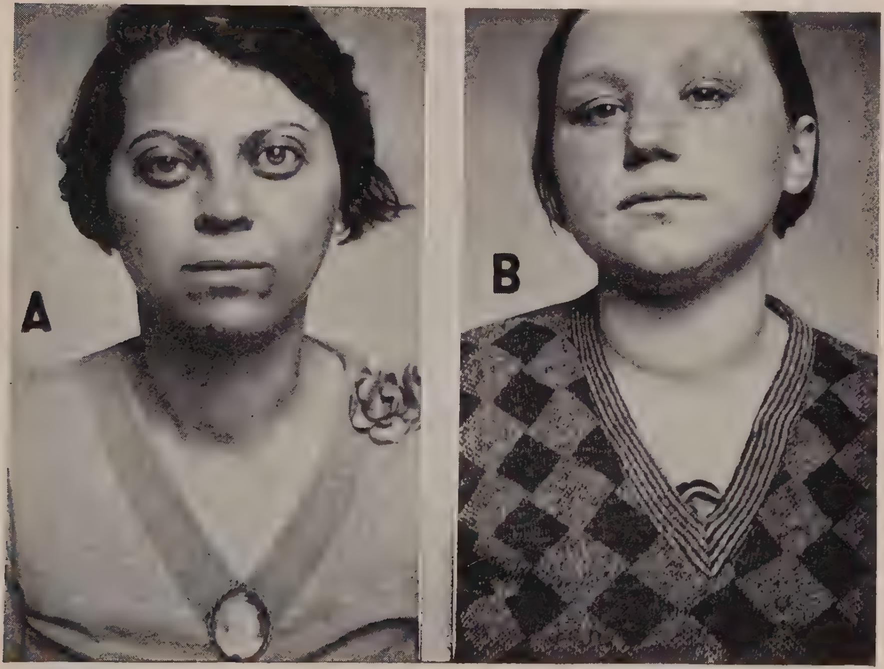

Fig. 57. A—H. W. with Marked Exophthalmos, yet Basal Metabolism is −9%.
B—S. R. with Prominent and Diffusely Enlarged Thyroid Gland, Whose Basal Metabolism is −4%.

These findings show that the state of the function of the thyroid gland cannot be determined by inspection alone.

Needless to say, general examination of the heart and vascular system, of all the organs that lend themselves to auscultation, percussion, and palpation must be practiced. Frequently an explanation of obscure symptoms will thus be discovered.

Type: Constitution

Besides the dissociated examination of individual factors and symptoms, it is necessary to classify a patient according to her leading characteristics and for this reason I regularly try to classify a given individual somewhat according to the following scheme. One of four main characters are exhibited by the average individual, who is not strictly abnormal or endocrine: (1) Feminine; (2) Infantile; (3) Neuter; (4) Virile.

The *feminine individual* is the normal woman fully described at the beginning of this chapter. Her form, her inclinations, her physiology, are all dominated by the feminine characteristics. In her, menstruation as well as childbearing usually run a normal course, unless disturbed by infection or neoplasia.

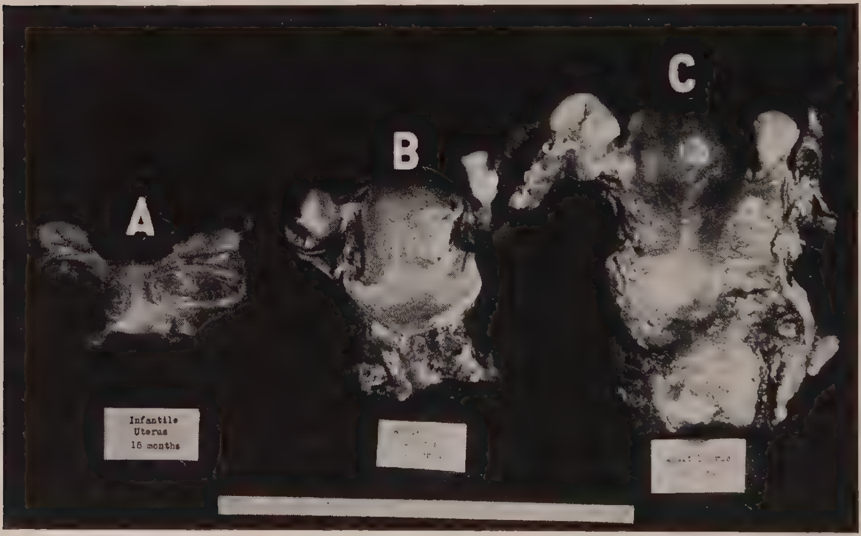

Fig. 58. Differences in Size and Proportion of Uterus at Different Ages.

A—Infantile (ratio between cervix and fundus 1:1). B—Adolescent (ratio 1:2). C—Adult (ratio 1:3).

The *infantile individual* never attains full maturity. Infantile proportions are recognized; the skin is soft and thin, the bones are light, the lower extremities short. The sex allures may be well developed; the secondary sex characters are rarely fully adult. The cardio-vascular system often is weak—"droplet" heart, low blood pressure, and asthenia are common. There may but need not be genital infantilism, with retention of the infantile cervico-corporeal ratio of 1:1 (instead of the adult 1:3). The vaginal fornices are often poorly developed. There may be general hypoplasia of the tubular system. Menstruation is commonly scant, irregular, and accompanied by pain.

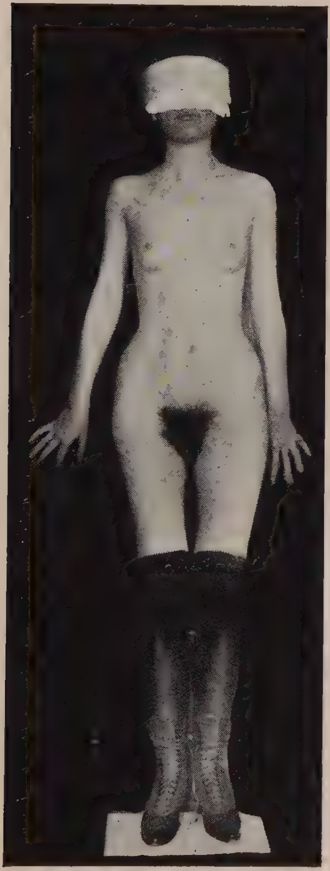

FIG. 59. FEMALE 23 YEARS OLD, EUNUCHOID TYPE.

Height 6 feet, 1 inch; long limbed; small larynx; high pitched voice; breasts, pelvis and hair distribution feminine. (From R. T. Frank: Gynecological and Obstetrical Pathology D. Appleton & Co., New York, 1922.)

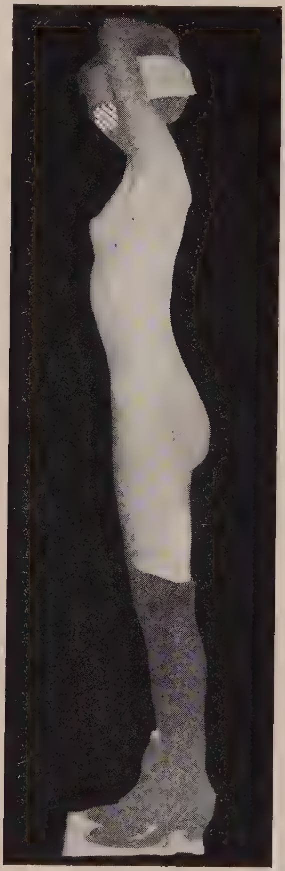

FIG. 60. SIDE VIEW OF EUNUCHOID FEMALE SHOWING LENGTH OF LIMBS AND EXAGGERATED SLENDERNESS.

The genital organs of this individual are normal.

The *neuter or eunuchoid individual* is long and thin. Her pelvis is poorly developed, inclining either toward the infantile or masculine type. The secondary sex characters are not fully developed. These individuals often are flaccid and poorly upholstered with fat. Their pelvic organs incline toward the hypoplastic, with poorly developed uterus, short fornices, short sacrouterine ligaments. Both this group and the infantile group are frequently the subject of dysmenorrhea, and include a large part of the non-mechanical sterile group.

The *virile female* is robust, florid, hirsute, hearty and muscular. Some individuals are frigid, others homosexual, still others oversexed and strongly heterosexually inclined. The pelvis is large, heavy-boned, inclined to the masculine, with high symphysis and narrow outlet. The clitoris is usually overdeveloped. The uterus may be well developed and normal. In some cases great fertility is encountered, but more often relative sterility is observed. These individuals incline toward hyperemia of the sex organs accompanied by menorrhagia and metrorrhagia.

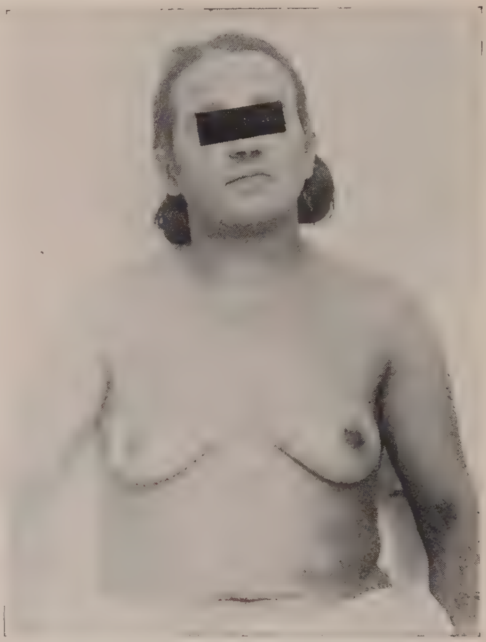

FIG. 61. MASCULINE TYPE OF WOMAN.
Broad shoulders; thick neck; hirsute body; mustached and bearded chin; heavy jaw; large ears.

Endocrine Deviations

In addition to actual endocrine diseases of a readily recognizable character, a number of individuals are encountered who show distinct evidence of past or present over- or underfunction of given endocrine glands. The resultant changes are recognizable but slight, and do not reach the degree of being classifiable as a real disease. I mention pituitary, thyroid, adrenal, and gonadic syndromes.

PITUITARY

Acromegaloid and adiposo-genital (Froehlich).

Acromegaloid. These individuals have prominent superciliary ridges, spacing of the teeth, long stature, big hands and feet, large viscera, spade shaped fingers, prognathism, to mention only a few striking symptoms.

The Froehlich type is short, soft-skinned, obese and flabby. Often the voice is high pitched and hair growth scanty.

Thyroid Type

Hyper- and hypo-functional.

Hyperfunctional types. These may conveniently be grouped under the guise of incipient Graves' disease, or exophthalmic goitre, and of the toxic type, both groups showing mild exophthalmos, slight tremor, slight tachycardia, occasionally looseness of the bowels, but little change in basal metabolism.

The hypofunctional myxedemoid group show coarseness of the skin, slightly sluggish reactions, supraclavicular fat accumulation and yet fairly normal basal metabolism. With time, true myxedema may supervene.

Of the *adrenal group*, I have only observed those with presumable hypoplasia of the adrenals, inclining towards pigmentation, asthenia, and low blood pressure. Fig. 62.

The *gonadic group* conveniently may be divided into oversexed and undersexed individuals. Among the oversexed are found exaggeratedly feminine types and a certain number of virile ones. The undersexed group is composed of the infantile and neuter, including the frigid, the sterile, the "dysmenorrhoic."

The Blood Count

Considerable information can be gained by routinely taking a blood count. This should include the hemoglobin determination and red blood cells, in order to differentiate the blood loss resulting in secondary anemia from that of primary anemia. The differential blood count is also of value both in regard to diagnosis, prognosis, and treatment. Leukopenia is occasionally encountered in functional bleeding; lymphocytosis above 30–35 per cent in the adult, is of distinct significance and import. Occasionally the blood platelets are reduced sufficiently to show that menorrhagias, for example, are due to a primary constitutional and not to a local condition. This applies as well to the bleeding time and coagulation time. Examination of the influence of the normal or abnormal cycle upon the morphology of the blood has given results too inconstant to be of value.

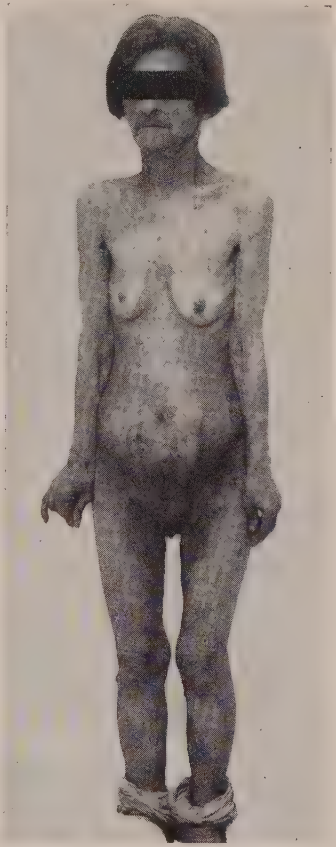

Fig. 62. Advanced Asthenia.

Infantilism and poorly marked secondary sex characters dating from childhood. In addition this unfortunate individual suffers from Von Recklinghausen's fibroneuromatosis.

The Basal Metabolism

The basal metabolism is of utmost importance. It is surprising how frequently neglect to perform this simple test as a routine measure, has resulted in serious error. Moreover, the fact that thyroid extract is the only reliable endocrine preparation, which we are as yet possessed of, for the treatment of functional ovarian conditions, makes it necessary to see whether, in the given case, this, our mainstay, may be safely given and in what dosage it can be borne. The fact that in many endocrine disorders as for example in both hyper- and hypothyroidism, menorrhagia, metrorrhagia, and amenorrhea may occur at different stages of the same disease, further proves the importance of determining the metabolism.

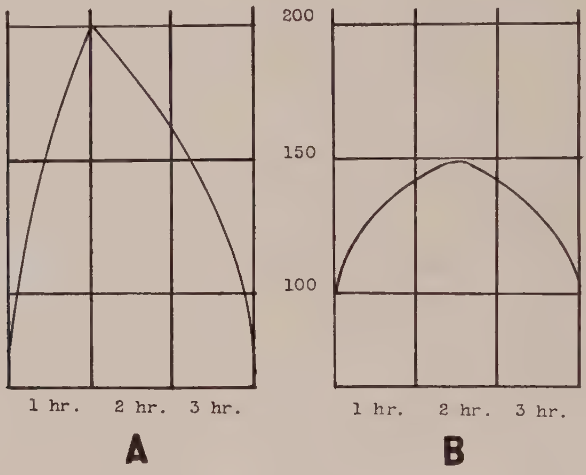

Fig. 63. Graph Showing Blood Sugar Curve as Obtained by Janney Test.

A—Normal tolerance with blood sugar rising to 200 mg. per 100 c.c. of blood in the first hour, with rapid descent to normal by third hour. Sugar appears in the urine by second hour.

B—Increased sugar tolerance. The curve is flat. The blood sugar rarely rises above 150. No sugar appears in the urine.

The Janney Test

The sugar-tolerance test is of great use, particularly in the presence of obesity. In pituitary disturbances and hypofunction of the ovaries, we regularly employ the Janney test, (Janney and Isaacson, 506) of which, for the sake of those interested, I give a brief outline as described by my collaborator, Dr. Gertrude Felshin Goldberger.

At a designated hour in the morning the patient presents herself after a twelve-hour fast.

1. 75 g. of glucose for each kilogram of body weight, dissolved in 300 c.c. of water flavored with lemon juice or citric acid, are given to the patient.

1. 0.2 c.c. of blood from the fasting patient is collected from a finger prick. A specimen of urine is collected.

2. The glucose is given.

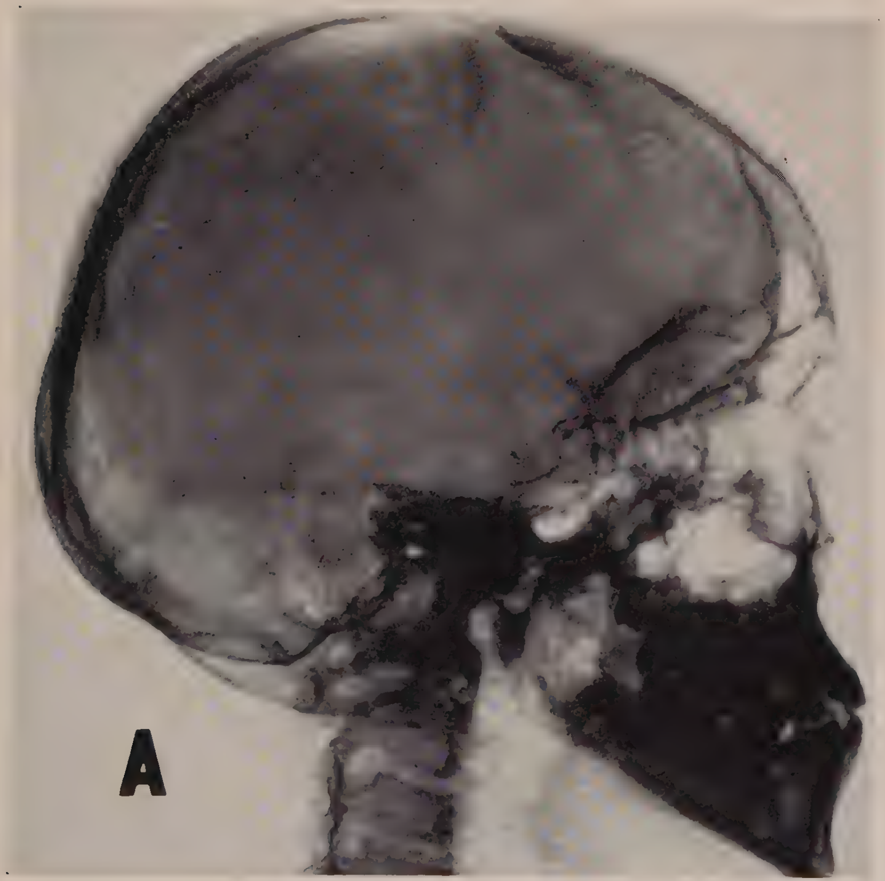

Fig. 64A. Sella Turica.
X-ray of skull showing normal sella turcica.

3. 0.2 c.c. blood one hour later. Urine collected.

4. 0.2 c.c. blood two hours later. Urine collected.

5. 0.2 c.c. blood three hours later. Urine collected. The blood sugar content of each of the four blood specimens and the presence or absence of sugar in the urine specimens is determined.

In a normal individual the blood sugar rises from the fasting level of 80 mg. per 100 c.c. during the first hour or hour and a half to 160–200. The second urine specimen contains from 1–2 per cent of sugar.

Increased sugar tolerance is recognized by failure of rise to a high level, a slow descent and absence of sugar in the urine.

(See curves, Fig. 63.)

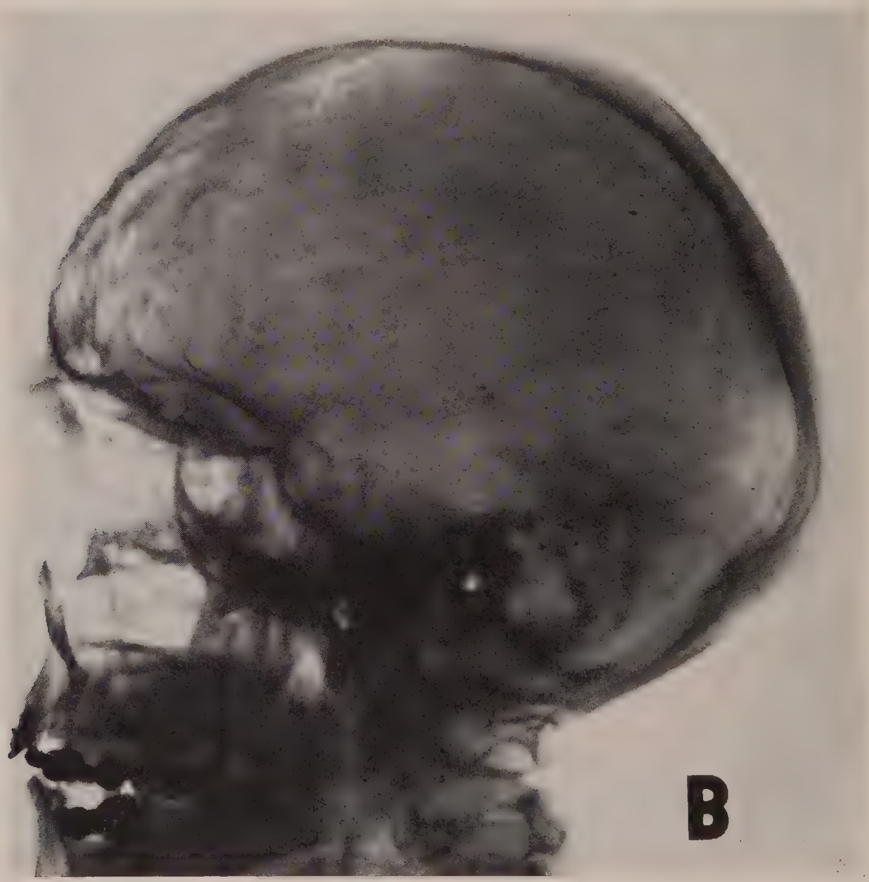

FIG. 64B. SELLA TURCICA.
X-ray of skull showing enlarged sella due to pituitary tumor.

Sellar X-Ray

In a large number of cases, study of the pituitary X-ray is indicated to determine whether the sella turcica is enlarged, normal, or small. Experience is needed to determine pathological conditions, as great variations in size are physiological. To make this examination worth while, the interpretation of an experienced radiographer is essential. The illustrations (Fig. 64) show a normal and an abnormally enlarged sella turcica. If the sella is abnormal, or visual disturbances have been complained of, a careful perimetric examination of the visual field is indicated. In cases of hypophyseal tumors contraction of the color vision fields must likewise be charted.

Testing of the Autonomic Nervous System

Although the earlier endocrinologists attempted to devise norms and tests for the autonomic nervous system, such efforts have largely failed. It has likewise proved futile to attempt to classify patients into vagotonic and sympathecotonic individuals because of the fact that in the same individual both of these deviations may be encountered. For similar reasons the testing of a response to given drugs such as pilocarpine, adrenalin, atropine, has proved futile and largely has been abandoned.

Surface Area

Recently, in our clinic, because of the interest shown by Dr. S. G. Berkow of Perth Amboy N .J., our patients who present abnormalities in the endocrine field, have been subjected to anthropometric measurement to determine their surface area proportions. Our results are as yet too few to be of real value but in my opinion have shown distinct promise. I therefore append a short abstract of the method as outlined by Dr. Berkow.

SURFACE AREA

by Dr. S. G. Berkow.

By means of the Linear Formula of Delafield du Bois and Eugene F. du Bois (497) the surface area of the seven natural divisions of the body is obtained. These consist of (1) head and neck, (2) the arms, (3) the hands, (4) the trunk including the genitals, (5) the thighs, (6) the legs, (7) and the feet. By addition the total body surface is obtained. Each of the seven areas gives a surface area proportion.

The accompaning figure shows the sites at which the measurements are taken.

The details are as follows:

HEAD: AB 0.308
  A—Around vertex and point of chin.
  B—Coronal circumference around occiput and forehead, just above eyebrows.
ARMS: F (G+H+I) 0.611.*
  F—Tip of acromial process to lower border of radius, measured with forearm extended.
  G—Circumference at level of upper border of axilla.
  H—Largest circumference of forearm (just below elbow).
  I—Smallest circumference of forearm (just above head of ulna).

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* Factor 0.558 if F is measured over olecranon with forearm flexed.

Hands: JK 2.22.

J—Lower posterior border of radius to tip of second finger.

K—Circumference of open hand at the metacarpo-phalangeal joints.

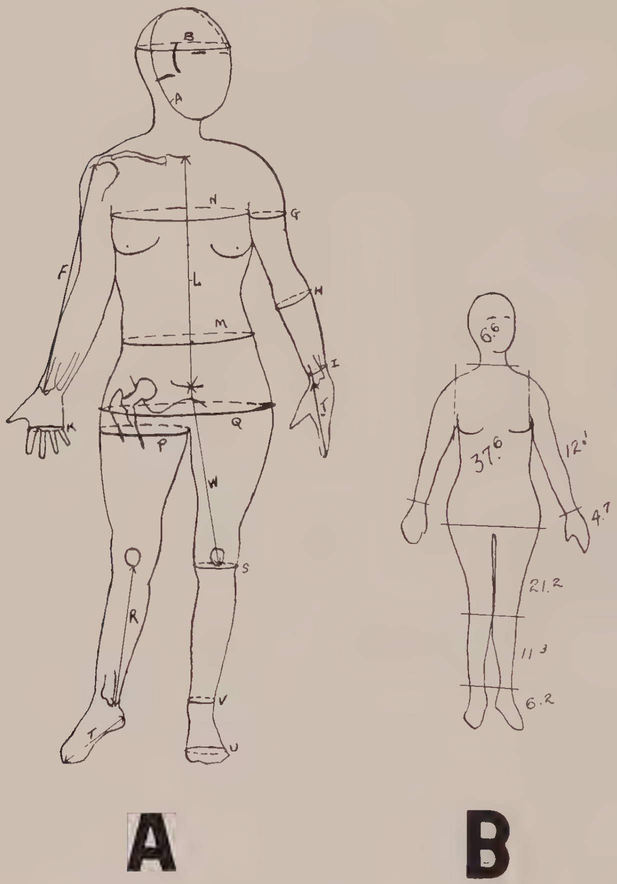


Fig. 65. Method of Obtaining Measurements of Surface Area.

Modified after Delafield Du Bois and Eugene F. Du Bois. A—Shows methods of obtaining measurements. B—Surface area proportions in normal individuals.

Trunk: (Including neck and external genitals in the male, breasts in female); L (M+N) 0.703.

L—Suprasternal notch to upper border of pubes.

M—Circumference of abdomen at level of umbilicus.

N—Circumference of thorax at level of nipples in the male and just above breasts in the female.

Thighs: W (P+Q) 0.552.

W—Upper border of pubes to lower border patella (measured with legs straight and feet pointed anteroposteriorly).

P—Circumference of thigh just below the level of perineum.
Q—Circumference of hips and buttocks at the level of the great trochanters.

LEGS: RS 1.40.
R—From sole of foot to lower border of patella.
S—Circumference at level of lower border of patella.

FEET: T (U+V) 104.
T—Length of foot including great toe.
U—Circumference of foot at base of little toe.
V—Smallest circumference of ankle (just above malleoli).

NOTE—The constants for arms, thighs, etc. when multiplied by the measurements of one side give the surface area for both sides. To find the total area add the seven parts.

Twenty-four cases of female sterility were studied by this method. Four sub-groups can be distinguished among the sterility proportions.

(1) 11 having normal female proportions.

(2) 6 resembling closely the proportions of the hypopituitary group.

(3) 3 whose proportions resemble closely the proportions existing in girls before puberty.

(4) 4 in whom the proportions correspond nearer to those of the adult male than those of the adult female.

It should be remembered that normal adult proportions exhibit but a narrow range of variation. Race affects the proportions but slightly. Nutrition affects them more, because the head proportions vary inversely to the state of nutrition, whereas the trunk proportions vary directly. Age too has influence on the proportions. At birth the head proportion is 18 per cent or about three times that of the adult. The proportions of the lower extremities vary in inverse ratio to the head proportions. For a given age, proportions are fairly constant, adult proportions being reached at about the 16th year.

FEMALE SEX HORMONE TEST

Upon individuals in whom abnormalities of the function of the ovaries were suspected, the female sex hormone test, according to our technic, has been performed. What follows in the succeeding chapters is based largely upon the results obtained with this test, although throughout we have kept in view the patient as a whole and not concentrated our attention either entirely upon her sex sphere or her hormone blood picture to the exclusion of all else.

CHAPTER X

HYPOFUNCTION OF THE OVARIES

A DIVISION into hypofunctional and hyperfunctional conditions due to a decrease or an increase of ovarian action is readily understood. The term dysfunction appears to be a misnomer, because no proof has been offered in connection with any endocrine gland that a change in the composition of the hormone secreted takes place. Oswald (518) agrees with my contention that no such phenomenon exists. Qualitative alteration alone would justify the term of "dysfunction." Clinically quantitative differences, differences in the time of occurrence of symptoms, differences due to the individual degree of reaction of the patient, may produce very diverse symptoms without requiring or justifying the concept of dysfunction.

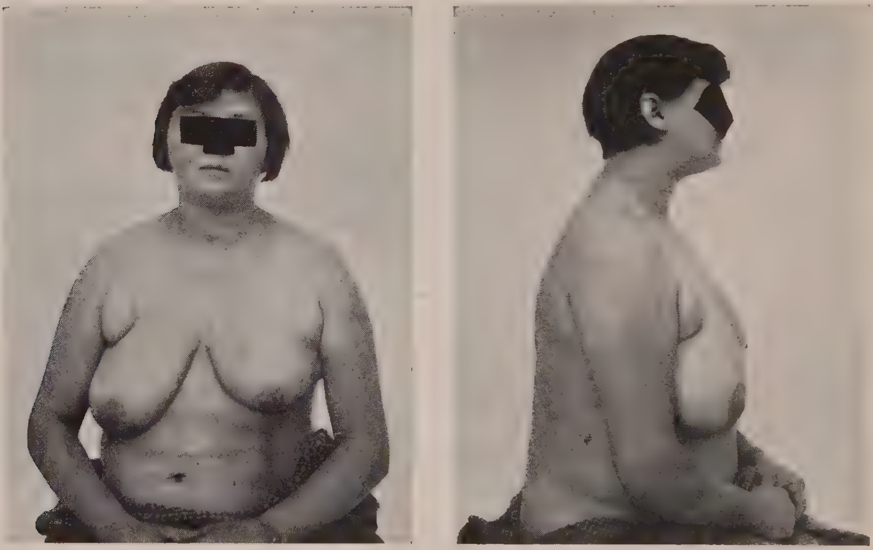


Fig. 66. Woman Showing Many Stigmata.

Small head; flat, small thyroid gland; right accessory breast; asymmetry of breasts; this ndividual is sterile, otherwise mentally and even physically well above the average.

Under the heading of hypofunction of the ovaries, in addition to a large number of women who appear constitutionally normal, three main types of handicapped individuals must be considered—the eunuchoid, the infantile, and the sterile. Frequently, though not necessarily, sterility is combined with one of the other types. In the vast majority of cases, one of the striking, if not the most striking symptom of this group of hypofunctional individuals is amenorrhea. Often it is easier to group these patients under the heading of this leading symptom, rather than according to the underlying causative factor which cannot always be determined. In this loose group, if amenorrhea has not as yet developed, oligomenorrhea, as well as dysmenorrhea are common symptoms. It is therefore of advantage first to discuss those patients whose leading symptom is amenorrhea. Very rarely indeed, a case of excessive uterine bleeding can be classified in the hypoplastic and hypofunctional group, the vast majority of these conditions occurring among the hyperplasias and hyperfunctional group.

Physiological Amenorrheas

In this discussion no attempt to deal with the physiological amenorrheas resulting from prepuberty, pregnancy, lactations or the senium will be made, our attention being focused upon those due to pathological causes.

Amenorrheas may be divided into primary and secondary amenorrheas.

Primary Amenorrheas

Primary amenorrhea embraces all patients who have never menstruated, although the time of normal puberty has passed. Those who can never menstruate because of anatomical defects, such as uterus rudimentarius solidus and absence of the vagina are considered under the heading of "Determination of Sex" on page 257. The second group, functional in nature, appears to possess a normally developed genital system which might be able to, and often later does, function normally.

Puberty without Menstruation

In spite of the absence of menstruation in these patients, puberty may manifest itself by every other sign, including the appearance of the secondary sex characters (hair, osseous pelvic development, fat distribution, voice, psyche) but without the accompaniment of menstruation.

Secondary Amenorrheas

Secondary amenorrheas manifest themselves by cessation of menstruation for longer or shorter intervals. The cessation is due either to underfunctioning of the ovaries, with scant production of female sex hormone, or although the production be normal in amount, the possibility of too rapid excretion from too low a renal permeability threshold for

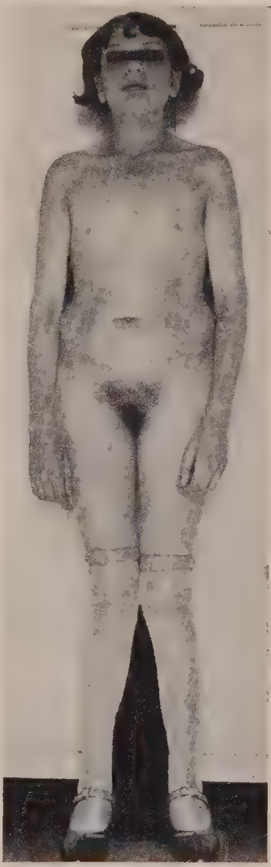


Fig. 67. Combination of Eunuchoid and Acromegaloid Stigmata.

Long limbs; large acra; vestigial breasts and nipples.

the hormone exists, the effect being the same in both instances. Among the causes which depress the ovarian secretion are:

A (1) General diseases such as tuberculosis, anemia.
  (2) Change of climate, as in the Irish immigrant girls.
  (3) Amenorrheas due to obesity.
  (4) Endocrine diseases, amenorrhea appearing at some stage of pituitary and thyroid hyper- and hypofunctional troubles, adrenal diseases and diabetes.

Other causes of amenorrhea include:

B (1) Unclassifiable causes. Among the latter are collected several groups. Some patients have a cycle, others no cycle. Some are self-limited (on the point of beginning to menstruate), and finally the rare persistence of a corpus luteum may cause amenorrhea.
  (2) Amenorrheas due to hysterectomy. In this connection we are interested mainly in the cases with the ovaries left *in situ*.
  (3) Amenorrheas due to castration, either X-ray or operative.

This study of amenorrheas is based largely upon class B 1 and A 3 of whom the blood has been examined in 47 cases.

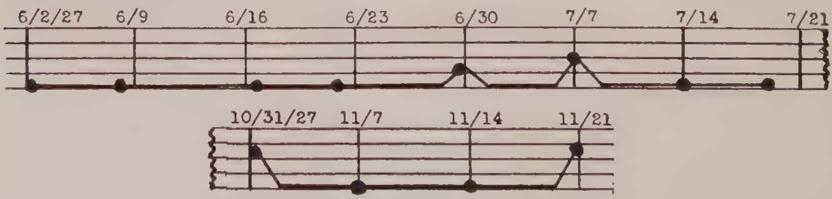
AMENORRHEAS

Primary Amenorrheas—Eleven Cases

Of this number, 4 showed some cycle, 4 showed a slight irregular cycle, and 3 no cycle at all. The seriousness of the condition must be judged by many factors, including the age of the patient, prolonged persistence proving the stubborness of the condition. The presence of many stigmata of endocrine or other disturbance adds to the gravity of the prognosis. Finally in those cases in which no female sex hormone blood cycle can be discovered, the outlook is worse than in patients in whom at least a regular subthreshold cycle occurs.

Patients with Cycle

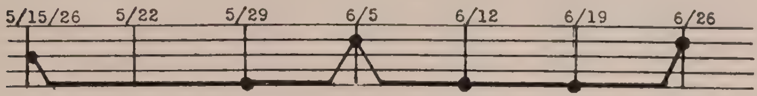
Of the 4 with fairly regular cycle, No. 1406, M.R., although with regular cycle, prognostically was graded as "poor" because of the markedly neuter physical development and the somewhat acromegaloid tendencies, both of which are readily recognizable from the illustration. (Fig. 67).



Graph 1406

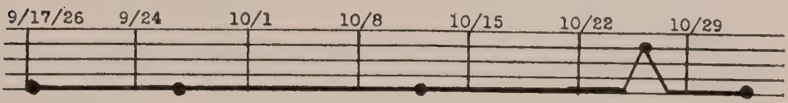
The other 3 patients, 1149, 1182, and 1391 showed few general abnormalities and hence are classified as having a more hopeful outlook.

No. 1149, R.M., 22 years, single, never menstruated. Feminine appearance.



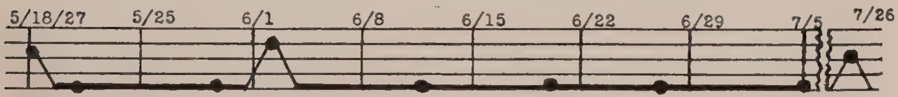
Graph 1149

No. 1182, 36 years, married, no pregnancies, never menstruated. Normal feminine appearance.



Graph 1182

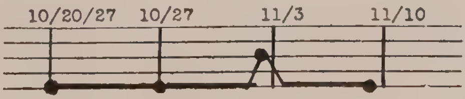
No. 1391, S. S., 22 years, married, no pregnancies, never menstruated. Feminine appearance. Basal metabolism −4 per cent.



Graph 1391

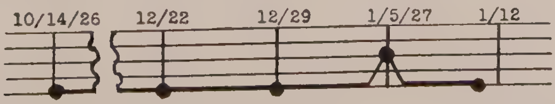
Patients with Slight Cycle

The 4 patients with slight cycles showed only an occasional rise above the zero level, and in general, may be regarded as extremely unfavorable cases. Of these cases, one was intellectually on a low level, had spaced teeth, was short in height, and somewhat overweight. (No. 1442).



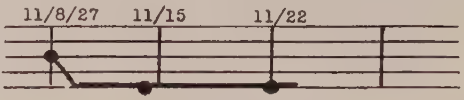
Graph 1442

Another, (No. 1192) although only 29 years of age, showed a very stubborn kraurosis vulvae which responded very favorably, but only temporarily, to the injection of an active preparation of female sex hormone and finally necessitated excision of the vulva. (See pages 270 and 283.



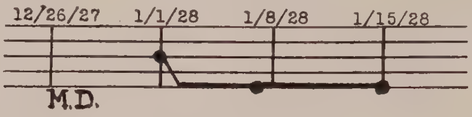
Graph 1192

Two other patients who presented no general abnormalities were also studied. No. 1475, H. B., 30 years, married, no pregnancies, never menstruated but had abdominal cramps every 28–30 days. The uterus was retroverted and admitted the sound 3 inches.



Graph 1475

No. 1531, M. C., 21 years, single, never menstruated. Normal feminine appearance.



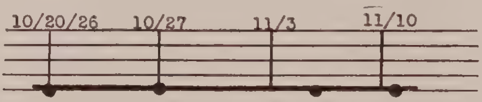
Graph 1531

Note:—M.D. signifies mouse died.

Patients with No Cycle

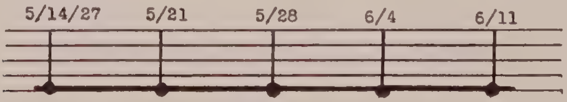
Of the 3 patients showing no trace of blood cycle, the one made a typical feminine appearance, No. 1204, with a slightly flattened pelvis, genitals in the infantile stage. This patient showed no cyclical reaction.

Filling of the breasts and attacks of abdominal cramps followed when 38 injections of an active preparation of female sex hormone were given, thus indicating that the infantile genitals responded readily even if not completely to the hormonal stimulus.



Graph 1204

The second patient, S. T., No. 1395, who likewise showed no cyclical blood changes, falls into the class of pseudo-hermaphrodites. In addition, distinct endocrine disturbances of acromegaloid type were noted.



Graph 1395

This strange looking person, as can be seen from the illustration (Fig. 68) had marked hirsutes. Her pituitary X-ray was normal, as was the basal metabolism. The body conformation verged toward the masculine. There were large hands and feet. The labia looked like a split scrotum. The vestibule in the prone posture was directed upward. The symphysis was beak-shaped. The vagina was funnel shaped; the uterus small in size. In spite of these well-marked stigmata, an experienced gynecologist performed a laparotomy on this patient and resected "large fibrotic ovaries."

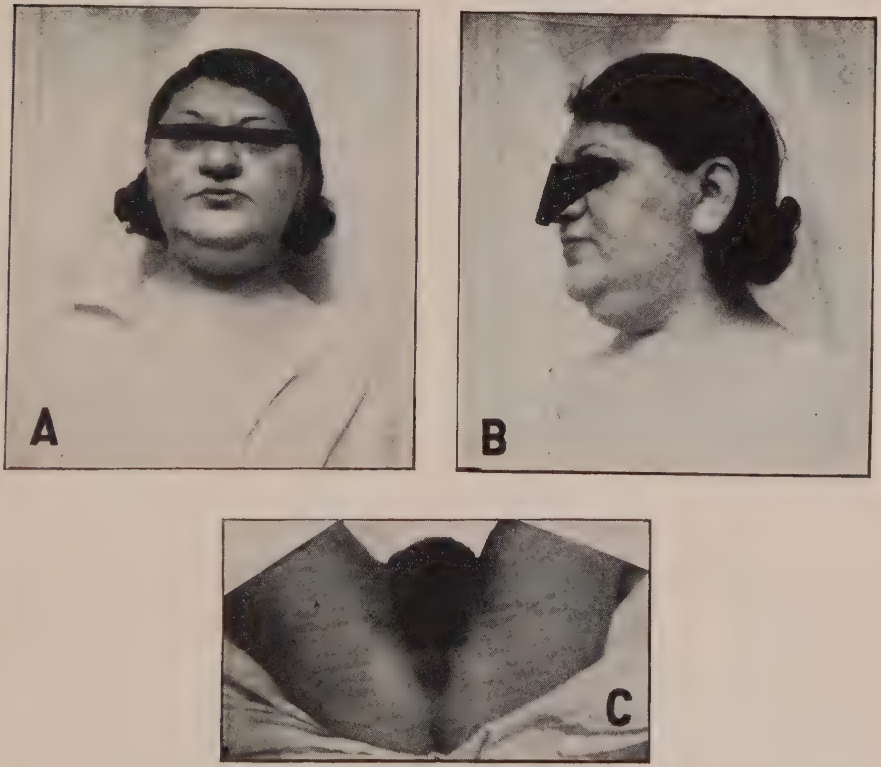
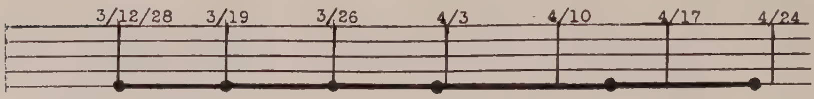


Fig. 68. Pseudo-Hermaphroditic, Acromegaloid, Hirsute Individual with Aplastic Genitals.

A—Front view showing broad nose; prognathism; prominence of superciliary ridges. B—Side view showing obesity; hirsutes; prognathism; large ear. C—Vulva with exaggerated size of mons, strikingly resembling a split scrotum. The miniature vulva and vagina are barely distinguishable.

Needless to say such an abnormal person should not have been operated upon, particularly as there was entire absence of blood cycle. I was unable to obtain the resected portions of the ovaries to inspect their histology, but would expect to find an ovotestis rather than a normal ovary in this individual.

No. 1615, P.L., age 26 years, married 1 year, nullipara; never menstruated, sex life normal. The patient is intermediate between the two preceding ones, not being as handicapped as the preceding patient but of typical eunuchoid type. She has mongoloid eyes, the upper and lower lips are heavy, the shoulders are heavy, the hips narrow, the arms and legs long, the feet and hands big. The uterus is small, pelvic outlet narrow; sellar X-ray normal; basal metabolism normal; sugar tolerance normal. As can be seen from the graph, there is no evidence of ovarian cycle. Prognosis is poor.



Graph 1615

Primary Amenorrhea and Pregnancy

Primary amenorrhea is not inconsistent with the possibility of pregnancy. In my experience I have encountered one woman who has never menstruated and yet has repeatedly had children. Another young Italian woman has menstruated 3 times in her life but has been unusually fertile. Her mother, too, menstruated only 2 or 3 times throughout her sex life, although she was the mother of a large progeny. (See Frank, 138). Through the kindness of Dr. Samuel H. Geist, the history of one of his patients is appended.

> Mrs. L. W. was seen first on July 17, 1925. She had no complaints but mentioned that she has not menstruated except one day at the age of 16 years. She was now 26 years old when Dr. Geist first saw her. She had been married one year and had had no children. Her physical examination aside from a somewhat undersized uterus, not infantile in type, and cystic prolapsed left ovary, was negative. She was a very tall woman; the exact height was not recorded, typically feminine in her build, with well developed breasts, and normal fat distribution. The only striking thing was a rather profuse hair development on her lips and cheeks and some coarseness of her features. Without treatment she became gravid and had her first baby on February 21st, 1926; the second baby she had on January 29th, 1928. The first baby she nursed for 8 months; the second baby she nursed for nine months. She is now gravid the third time, about 6 weeks.

Similar cases are reported by Hoover and Marden (504) and Blankengship (492). The former authors reported a woman of 40 years who had never menstruated and had borne 11 children.

SECONDARY AMENORRHEAS

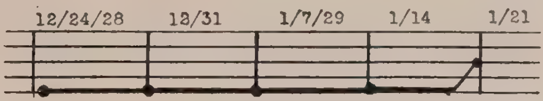
The secondary amenorrheas, for the sake of simplicity, for the majority defy any attempt at etiological classification, have been divided into two groups, simple secondary amenorrheas with various lengths of duration, and obesity amenorrheas.

Secondary Amenorrheas of Long Duration (one to fourteen years)

In this group I have placed 8 cases. The duration in itself does not signify anything as to prognosis. The history of previous pregnancy, other things being equal, improves the outlook. The amount of blood reaction noted is of great significance, the prognosis being better in patients in whom some cycle can be demonstrated. The presence of severe congenital or acquired endocrine stigmata adds to the poorness of the outlook.

It is of interest to note that in none of this group was any real or permanent improvement obtained by means of therapy, in marked contradistinction to the obesity group later to be referred to.

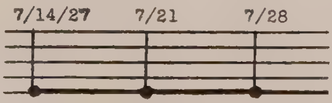
No. 1734, J. J., 29 years of age, was a patient with 14 years of amenorrhea, in whom a pituitary tumor had been removed a number of years ago. In her case the blood reaction is extremely weak and irregular.



Graph 1734

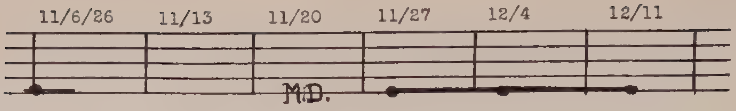
The long duration and early onset of the symptoms may be judged from the fact that Dr. Berkow's anthropometric measurements put her unmistakably into the hypopituitary group. Unless the future yields us an active anterior pituitary lobe product, the outlook for this patient is absolutely bad.

No. 1427, E. F., only 17½ years of age, falls almost as well into the group of primary amenorrheas, having menstruated only irregularly a few times between the age of 12½ and 13½ years, and is now amenorrhoic for 4 years. Her basal metabolism is −13 per cent. She is sluggish, with mental depression. Yet this patient does not respond well to thyroid therapy. There is no blood reaction. This patient does not belong in the group of transient amenorrhea of adolescents, frequently occurring at the ages of 13-17 years or even later. In these the condition is not serious or permanent.



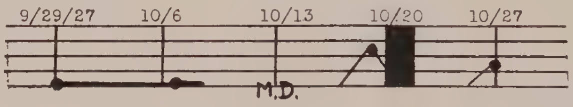
Graph 1427

The case of No. 1227, I.S., 29 years, is typical of misdirected therapy. Amenorrhea has existed for 6 years. The patient complains of sterility and amenorrhea. There is no blood cycle. One year ago radium was inserted for 9 hours into the uterus. The exact amount used could not be elicited. Evidently it was hoped to "stimulate" the ovaries by radiation. In our opinion the slight chance this patient had was irrevocably jeopardized and destroyed by this rash intervention.



Graph 1227

No. 1435, H.K., in whom the amenorrhea has lasted for 2 years, appeared to warrant a much better prognosis. Twenty-four years of age, with one child, six and one-half years ago, this patient showed a slight but irregular blood reaction. She complained of flushes, seemed generally normal, and when, on the second blood examination, a 2+ was found, an excellent prognosis was given.

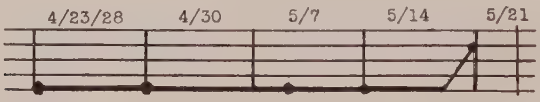


Graph 1435

Both to the patient's and our surprise, this surmise was immediately confirmed by the fact that a menstrual flow occurred 4 days after the blood was taken.

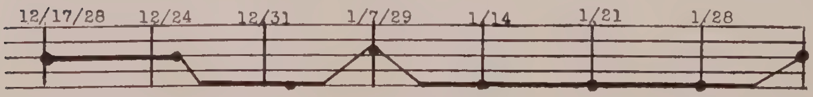
The accompanying short outline, together with the graphs illustrating the blood cycles or absence of them, sufficiently describes the other 4 cases.

No. 1654, E. P., 28 years old, married, 0 pregnancies; amenorrhea 8 years; B.M. −13 per cent, sellar X-ray normal.



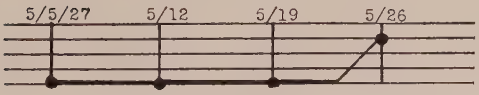
Graph 1654

No. 1730, F. K., 31 years, 11 para, amenorrhea six and one-half years, menstruated once, two months post partum (six and one-half years ago); B.M. +23 per cent, no thyroid symptoms, sugar tolerance normal; weight 121 pounds, normal. Fairly regular subthreshold cycle. Prognosis good.



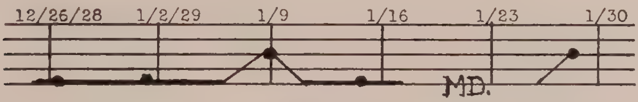
Graph 1730

No. 1387, N. N., 16 years old, began at 12 years, always irregular; amenorrhea for one and one-fourth years. Began to menstruate after 2 months and is now regular.



Graph 1387

No. 1735, A. S., 27 years, single, amenorrhea, one and one-half years.



Graph 1735

SECONDARY AMENORRHEAS OF SHORT DURATION
(TWO TO TEN MONTHS)

This group comprising 13 patients, on the whole, gave a much better prognosis than the preceding one. Of 7 cases over 6 months in duration, 4 showed a subthreshold cycle. Of 6 cases with less than 6 months of amenorrhea, on the other hand, only 2 showed any cycle. In 2 of these patients, the question of pregnancy was posed because of the short duration of the amenorrhea and the youthful age of the patients. In both a negative blood reaction showed that pregnancy did not exist. In 2 of this group, because of their more advanced age, the question of impending menopause was raised. In one of them, 41 years of age, a 0 blood examination proved the absence of pregnancy. In the second, 45 years of age, a 3+ blood reaction lead me to diagnose pregnancy, which later was verified.

In 4 of these patients in whom a high concentration of hormone was found in the blood, impending menstruation was predicted. The following abstract will show how fully this prediction was justified.

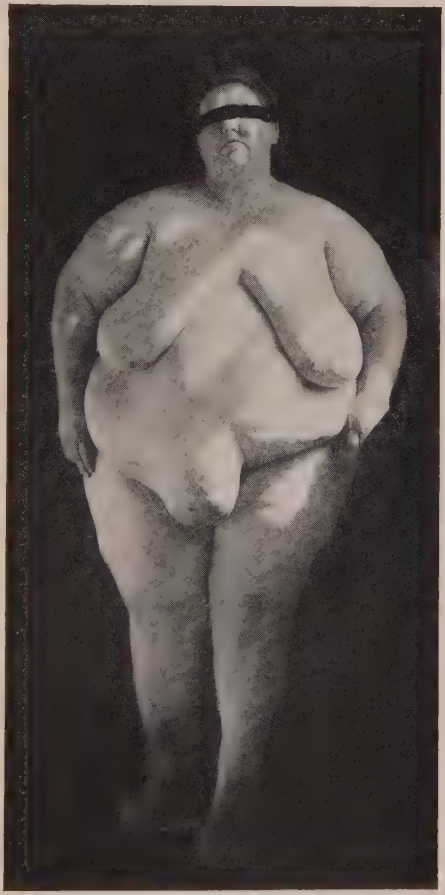


FIG. 69. DIFFUSE OBESITY OF EXTREMEST GRADE.

Weight 405 pounds; marked fertility; 10 children; abdominal hernia due to operation.

Case 1258—2 months amenorrhea—blood +3 bled next day.
" 1165—5 " " — " +3 " same day.
" 1206—8 " " — " 2+ " next day.
" 1435—1½ years " — " 2+ " 3 days later.

OBESITY AMENORRHEAS—TEN CASES

Of the patients studied the heaviest weighed 414 pounds, the average was 200 or more pounds. Of these patients 6 showed no blood cycle, 3 showed a weak, irregular blood cycle. In one patient, although the cycle was irregular, the high level of +3 was reached.

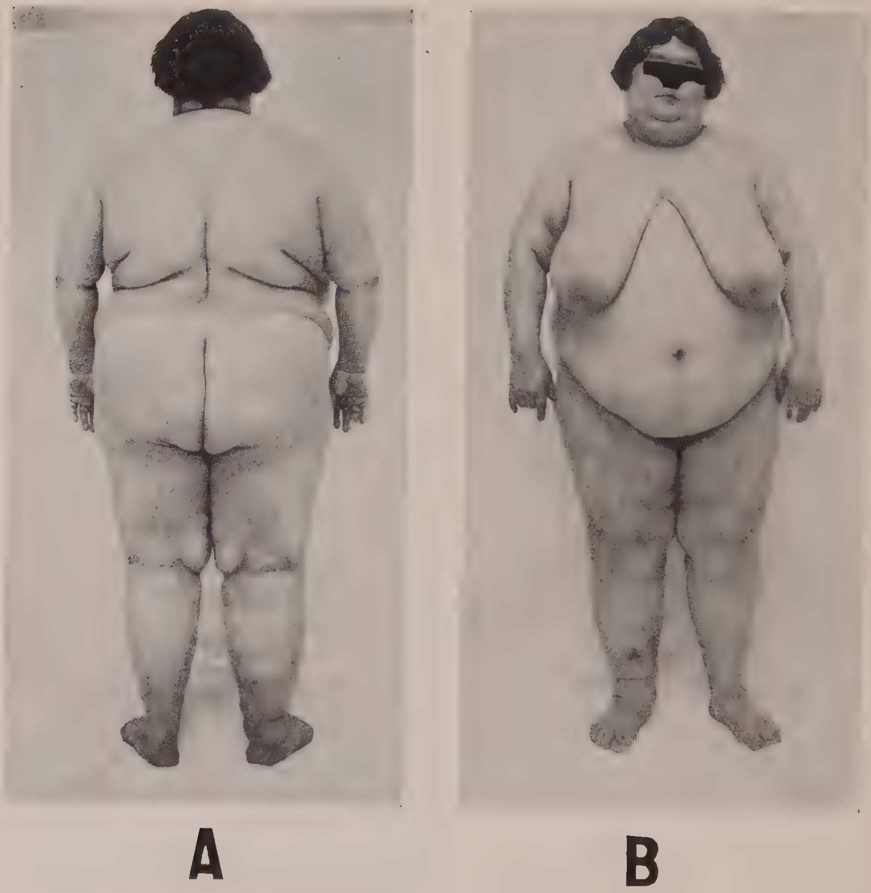

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Fig. 70. Diffuse Obesity.
275 pounds; fertile; now amenorrhoic. A—Back; B—Front.

Obesity Amenorrhea
With Good Cycle

No. 1188, F. R., 26 years, married 8 years; 2 children; weight 225 lbs.; amenhorrhea 5 years but menstruated 5 days before being seen.

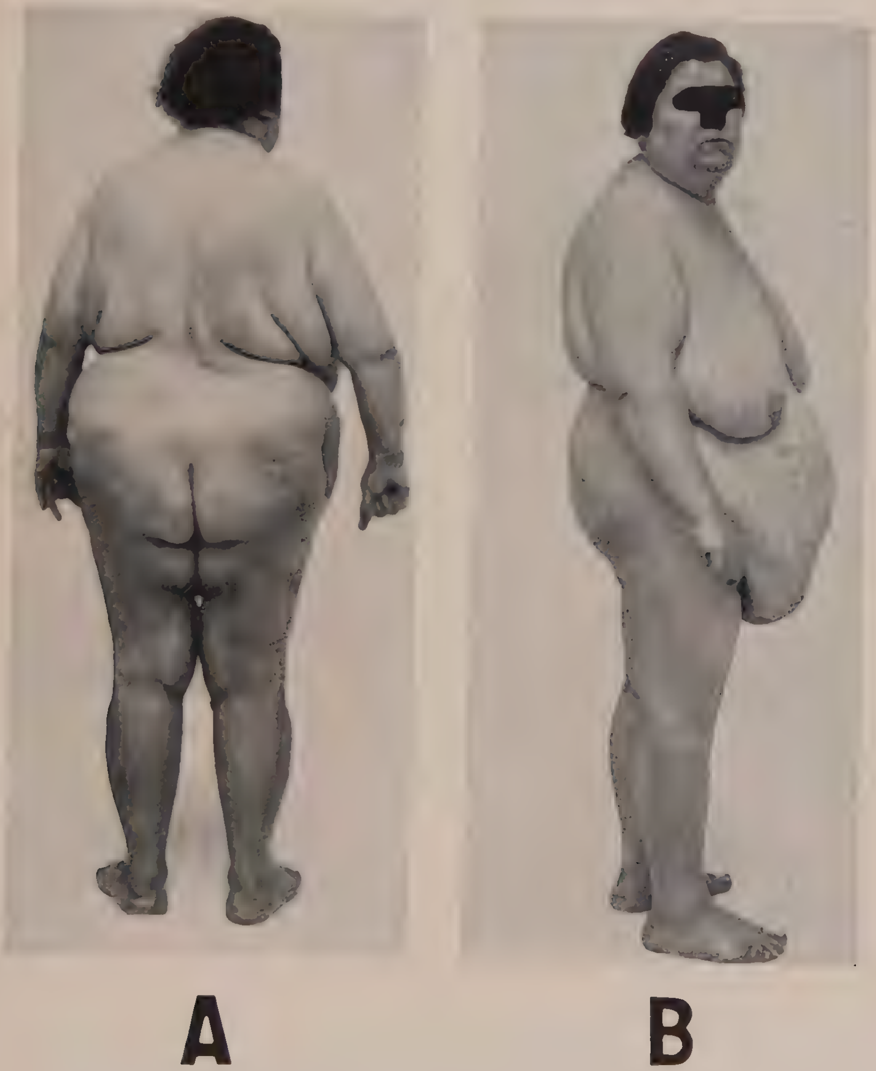
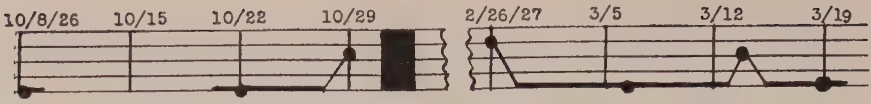


FIG. 71. DIFFUSE OBESITY.

Lower limbs not affected. A—Rear view; B—Side view with apron-like projection of abdominal fat.

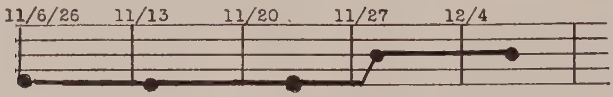
Basal metabolism normal. From the history alone a good prognosis could be given. Loss of weight re-established normal function.



Graph 1188

With Weak Cycle

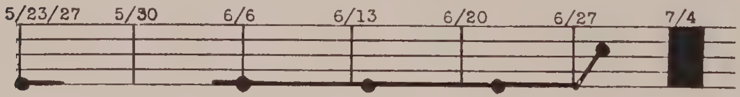
No. 1228, E. R., 18 years old, married 3 years; no pregnancies; 5 feet tall, weight 200 pounds; amenorrhea of 13 months followed by one period 9 months ago; large clitoris, uterus normal.



Graph 1228

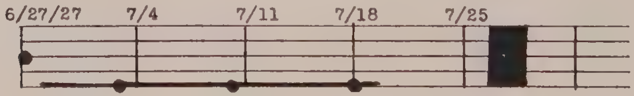
No. 1398, M. C., 33 years, married 18 years, 8 para; weight 255 pounds; has gained 60 pounds in last year; amenorrhea 14 months; sellar X-ray negative; fully feminine.

By diet and thyroid medication this patient lost 75 pounds. Her menses became normal; in September 1928 she conceived.



Graph 1398

No. 1417, A. A., 29 years, married two and one-half years, 0 para; gained 40 pounds in last two and one-half years; some facial hirsutes; amenorrhea two and one-half years. Basal metabolisn −2 per cent; sugar tolerance normal.



Graph 1417

With No Cycle

Six cases were studied (No. 1401, 1429, 1450, 1486, 1583, 1656). One of these patients weighed 414 pounds. The lightest weighed 175 pounds; all the others over 200 pounds. Of the six, three became regular in menstruation upon thyroid therapy and reduction of weight.

These obesity amenorrheas, in most instances, did not show a notable reduction of the basal metabolism. In most of the patients the obesity was diffuse, but in several instances typical "girdle" obesity was noticed (see fig. 72). In one patient a marked "shoulder girdle" obesity had developed, which one of my assistants strikingly described as the "prize fighter" type of obesity. (See fig. 73).

Those patients who could tolerate moderate doses of thyroid substance and who cooperated sufficiently by careful diet to lose a pound a week, responded gratifyingly to these simple measures. After the loss of 25 to 40 pounds, normal menstruation was noted in a number of instances.

Amenorrhea due to endocrine causes will be considered under various headings.

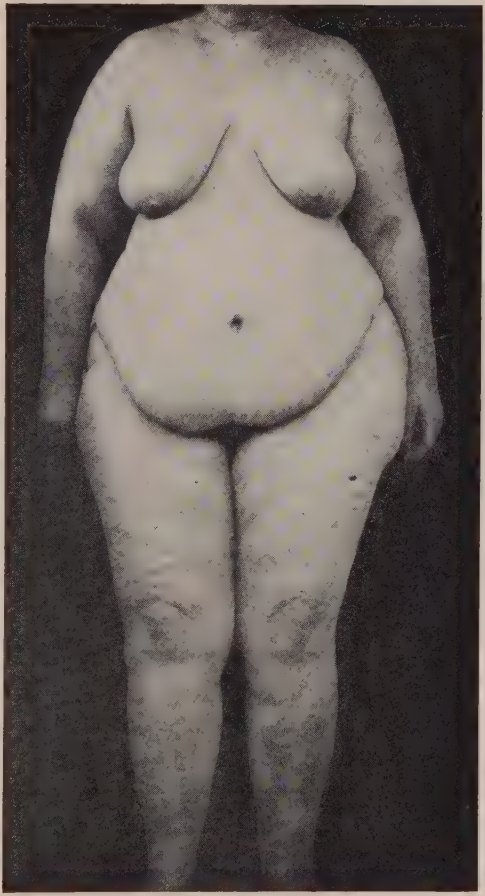


FIG. 72. TYPICAL LOWER GIRDLE TYPE OF OBESITY. Woman approaching menopause.

Amenorrhea Due to Hysterectomy

The blood was studied in three cases in which the ovaries were left *in situ*. This interesting group showed that the ovaries may function normally after removal of the uterus. They also indicate that a threshold mechanism other than that resulting from uterine excretion, exists. For details see p. 256.

Amenorrhea Due to Castration

The blood of operative castrates we have not examined as a routine. On the other hand, we have repeatedly watched the blood changes in patients subjected to X-ray castration. Both in the pregnant (see p. 252) and the non-pregnant, the effect became manifest in from one to three weeks. Contrary to the reports of Brambell and Parkes as observed in the vaginal cycle of the mouse (55) no cyclical blood changes were observed in the human unless less than a "sterilizing" dosage was administered.

After the X-ray effect has worn off, and this period varied between four months and one and one-half years, the blood cycle reappears before the menses are re-established.

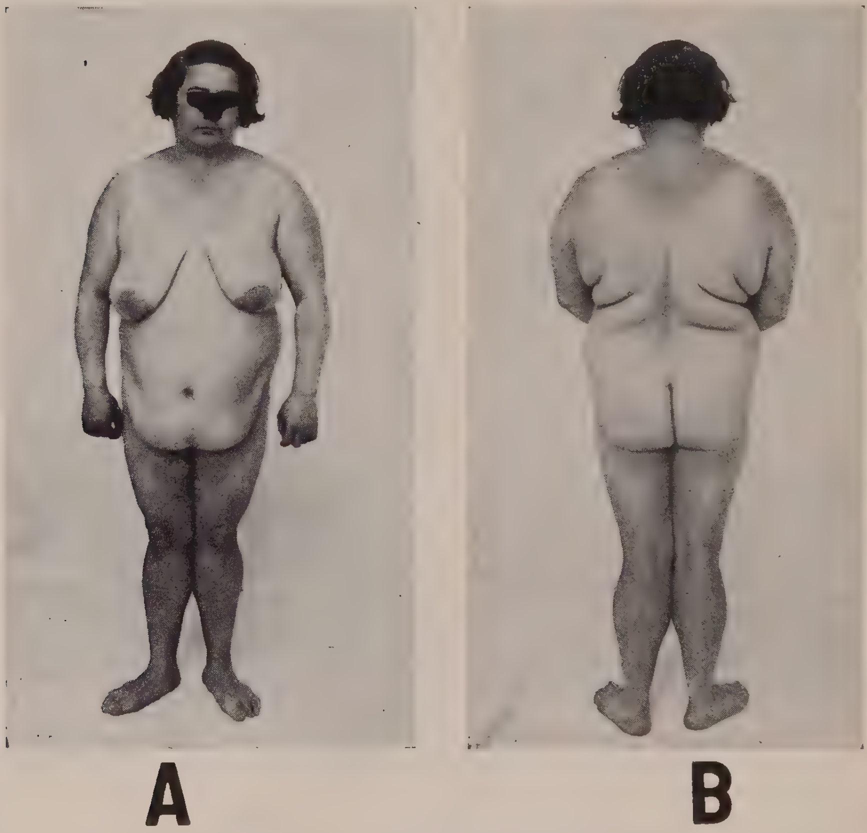


Fig. 73. Rare Upper Girdle Obesity.

A—Shows broad shoulders, pendulous breasts, apron-like abdominal fat, thin hips and lower extremities. B—Shows the upper girdle obesity to an even more striking degree in this same individual.

Amenorrhea Due to Persistent Corpus Luteum

Neither the work of Reynolds (388) nor of Ochsner (517) who spoke of amenorrhea in the human being due to persistent corpus luteum is convincing. The amenorrhea of early pregnancy apparently is due to the persistence of the yellow body (see page 54) but in our human material we can offer no proof of this.

On the other hand, a single clear-cut case occurred in our experience which apparently belongs to the group in question. A patient who was in the hospital under observation for a long period of time for a pelvic inflammation, passed her period and throughout the next four weeks during which her blood was carefully and repeatedly studied, the hormone level was found to be a 4. No opportunity for pregnancy had occurred, nor did subsequent observations yield a different diagnosis, as after the 4 weeks of amenorrhea, the blood dropped to 0 and eventually cyclical menstruation was resumed. Because of the constant high level in the circulation, we feel justified in considering this a case of amenorrhea due to the corpus luteum. In those cases in which persistence of follicles is noted, bleeding takes place, as has been shown by Aschner (27), Schröder (407), and Geist, (176).

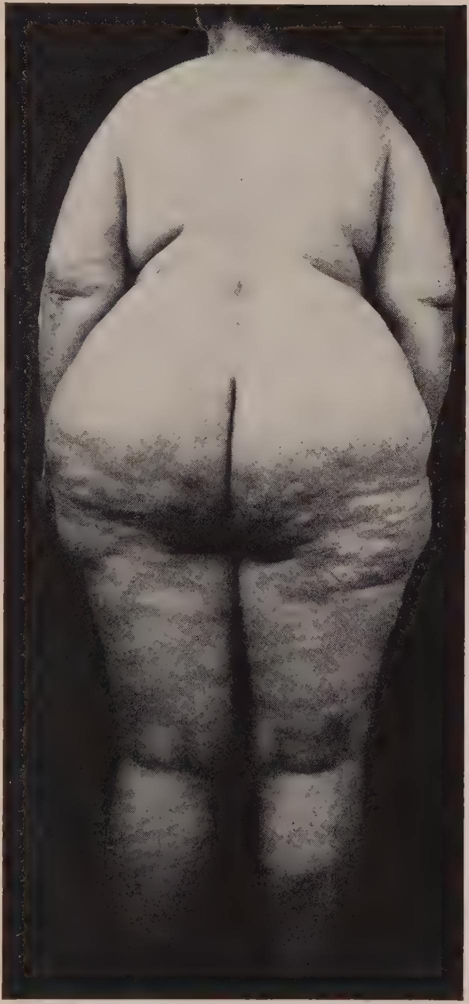


Fig. 74. Adipositas Dolorosa.

Woman 59 years of age; weight 220 pounds; basal metabolism normal. Each fat nodule is intensely sensitive to pressure. This patient cannot lie in the same position for more than 5 minutes. No relief obtained.

SUMMARY

Analysis shows that the study of the blood cycle is of utmost value for grouping and prognosticating in conditions of amenorrhea. Although other factors like obesity, evidence of such diverse endocrine symptoms as hirsutes, prognathism, asthenia, and pigmentation must be considered as well as developmental conditions, such as well-marked infantilism or eunuchoidism, the final decision of the gravity or transient nature of the condition will rest on the determination of the hormone blood picture. It should be recalled that in 4 cases varying from 2 months to one and one-half years in duration, the high level found in the blood, lead us to predict the early occurrences of menstruation, which actually took place within less than 3 days in each instance. In these cases the results would have been ascribed to any therapy which might have been instituted in these patients unless controlled by our blood test. On the other hand, a still higher blood hormone level indicates a pregnancy of more than 7 weeks in duration (see also p. 248).

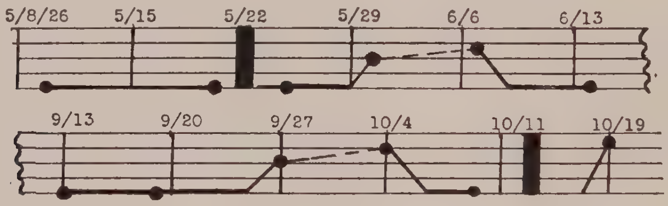
Oligomenorrhea and Irregular Menstruation

From the few cases examined, it appears that when the menstrual cycle occurs at long intervals, or when the bleeding is scant (oligomenorrhea), the blood cycle may be more regular than the menstruation would indicate.

Premature recurrence of the acme of a blood cycle was encountered in one case for which we can offer no explanation as yet.

No. 1397, S.S., 22 years of age, married 3 years, nullipara, normal except for male suprapubic hair distribution and −9 per cent basal metabolism. Very infrequent and irregular menstruation, the bleeding occurring every 3 to 4 months.

Repeated examinations of her blood showed an anomalous rise of the hormone level in the circulating blood shortly after the completion of the menses. Whether this signifies over-hasty but incomplete ripening of follicles, I am unable to determine as yet.



Graph 1397

STERILITY

A large number of sterile patients whose husbands had been found potent,* in whom no inflammatory cervical plug (endocervicitis) was present, whose tubes were found patent, presented themselves for study. A number were grouped under various headings such as endocrine disturbances, infantilism, amenorrhea, irregular or scant menstruation, etc. Comparatively few were free of both serious endocrine stigmata and had a regular menstrual cycle.

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* To my surprise, women are being increasingly subjected to study for fertility, including insufflation of the tubes, without the husbands' semen having been first examined. As at least 30 per cent of all sterility can be ascribed to the male partner, this omission appears inexcusable.

This small but important aggregation of carefully studied married women was scrutinized with the hope of eliciting additional information. In 22 cases, 57 bloods were tested.

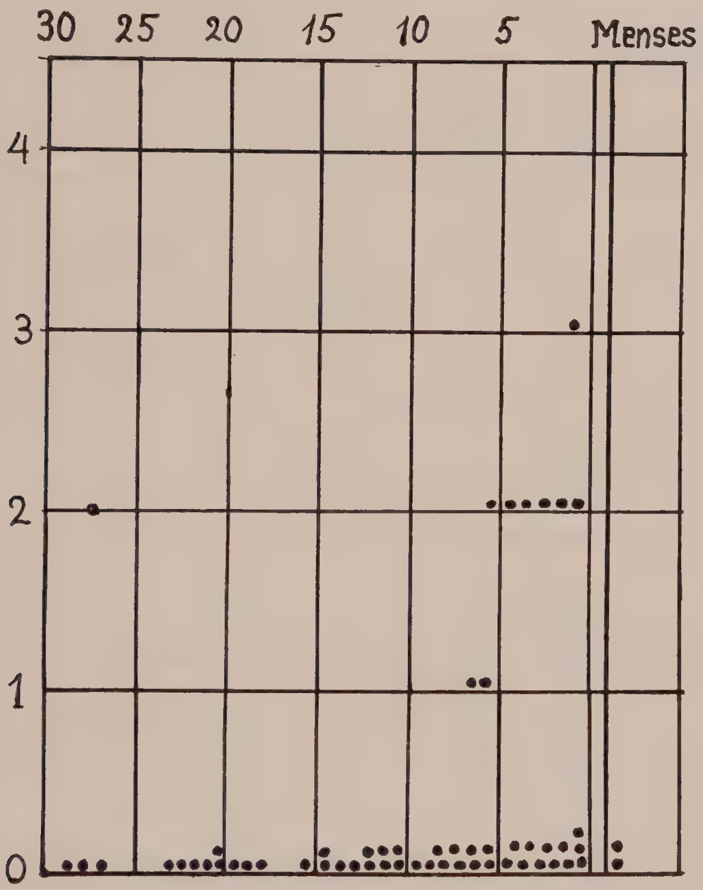


Fig. 75. Chart of Sterility Bloods.

Chart showing venous bloods in sterility cases. Note low premenstrual threshold which may be of significance in explaining failure of imbedding of ovum.

The striking finding is the uniformly low premenstrual threshold level. In 18 bloods taken between the 5th day before and the day of menstruation, only one reached a full threshold level and but 6 reached the +2 (weak, ±, doubtful) reaction. The contrast of this with the normal level of the most carefully selected group of "Norms" (Fig. 52) is striking and perhaps significant. Tentatively I suggest that these sterile women have a low female sex hormone permeability threshold which does not allow the uterus to attain the pregravid endometrial change necessary for nidation.

If this holds true, fertilization of the ovum would nevertheless not lead to imbedding of the fertilized ovum, which, without being able to obtain a foothold in the uterine mucosa, would be aborted at the earliest stage, in spite of the sensitizing action of the corpus luteum. This can be assumed to be so, for the experiments of Weichert (p. 52) have shown, that unless the uterus reaches a full estrous reaction, the corpus luteum deciduoma (placentoma) effect cannot be obtained. The deciduoma represents the maternal portion of the placenta.

With the comparatively scant data on hand, for the present, I prefer evaluating the gravity of sterility by other data rather than those obtained by our test.

The amount of female sex hormone excreted in the urine requires study. A continuously high and increased urine level was noted in the only case of this class in which the urine was investigated.

For Graph 1392 see Fig. 54, B, page 191

The blood hormone level in this patient has always been low, never rising above 2 premenstrually.

As I have emphasized previously (Frank, 150) women may be divided into

(a) abnormally fertile
(b) normally fertile
(c) temporarily sterile–lactional atrophy
    –physical depression
(d) permanently sterile–congenital
    –early acquired (stigmata)
    –late acquired (hypoplasia)

Each of these groups requires detailed study before the problem can be fully understood.

Eunuchoids

The typical eunuchoid or neuter individual is "subfeminine." These patients are tall, bony, of the "old maid" type. The pelvis is narrow, the subpubic angle acute, all pelvic measurements contracted. The feminine secondary sex characters are poorly developed. (See male breasts of Fig. 67, p. 213.)

The two most striking cases observed by me were in patients who had never menstruated (Nos. 1406 and 1615, pp. 214 and 217). Only one showed a blood cycle. The typical eunuchoid seen in Fig. 59 on the other hand, menstruated regularly and conceived.

It follows that very varying degrees of eunuchoidism are encountered. The condition *per se* need not necessarily imply severe disability of the sex apparatus. In this group, however, hypoplasia, amenorrhea and sterility predominate in the symptomatology. For details see Tandler and Grosz (437).

INFANTILISM

Minor degrees of infantilism are very common and may be limited to individual organ systems, such as the skin, hair, and osseous skeleton or to the genital tract. In well-marked general infantilism, both the body, the sexual organs, and the psychic equipment are affected. Most commonly infantilism is associated with general hypoplasia. Frequently heredity plays a rôle.

These individuals make the impression of never having grown up. They are usually slight in stature with light bones, short lower extremities, in contradistinction to the eunuchoid whose extremities are unusually long. Their pelves are narrow, often generally contracted, the sub-symphyseal angle being acute, the promontory high. A regular stigma is the very high palate arch. The teeth are frequently defective in their enamel. The secondary sex characters of these individuals are usually poorly developed. The hair is often very fine but scanty. Lanugo may persist after puberty has been reached. The heart is usually small, often droplet-shape and the aorta narrow. The larynx is high and small. The breasts are poorly developed or vestigial. The nipples are often retracted.

The vulva is funnel shaped. The labia are small, the perineum rigid. The vestibular area is prominent. The vaginal fornices are usually short and undeveloped. The portio vaginalis is either long and spindle-shaped, or extremely small and hypoplastic. The corpus uteri is unduly slender and flabby and frequently a sharp anteflexion results from the shortness and inelasticity of the sacro-uterine ligaments. The increased convolution of the tubes, the changes in consistency and shape of the ovaries described by Herrmann (503) are not elicited by pelvic examination. Frequently Douglas's cul de sac is unduly deep, forming a narrow, fingerlike projection in the recto-vaginal septum. The bladder may be narrow and spindle-shaped, being extrapelvic in location, as in the fetus.

These individuals show poor resistance to disease, withstand the traumata of life poorly, and may be sexually frigid and prove both sterile and amenorrhoic. On the other hand, many of these infantile women, because of their vivacity, petiteness, soft skin, and clinging qualities, are attractive. They marry early but prove poor wives as they readily become neurotic and so often are sterile. Chrobak and Rosthorn (494) describe infantilism with a great deal of detail. According to these authors, infantilism occurred in 3 per cent of 2500 unselected cases. On the other hand, as high as 25 per cent has been reported by some authors in their gynecological patients. Our tests have not been applied to cases which could be classified simply as infantilistic and therefore we have no additional data to give. What has been said of the amenorrhoic and sterile group applies as well to the infantilistic individual.

IRREGULAR BLEEDING DUE TO HYPOFUNCTION

In my experience the vast majority of menorrhagias and metrorrhagias are of hyperfunctional origin. In my entire investigation only four cases in which the opposite seemed to obtain came under observation.

The first patient was seen and described by me in 1914 (Frank, 138, footnote 86). This patient had bled continuously since the onset of puberty. She was 28 years old when seen. At that time she was obese, had scant hair, an infantile pelvis and small thyroid. Leucopenia and relative lymphocytosis were noted. The genitals were slightly hypoplastic. Only during the last three months had some semblance of periodicity been re-established.

The second was studied in 1923. A young thin-boned but obese girl of 17 years (weight 240 pounds) who had bled continuously for two years (see Fig. 76). Curettage showed resting endometrium and afforded relief only temporarily.

The third was a patient who had been bleeding steadily for 5 years, in spite of curettage and all medical measures which were tried. Her condition is not clear-cut because other endocrine elements exist. The patient, as can be seen from the accompanying illustration gives a "queer" impression. She does not fit entirely into the acromegaloid class but shows many of these stigmata. When first observed she was much overweight, pudgy, and was suspected of being myxedematous. However, the basal metabolism was approximately normal. Dorso-cervical lordosis existed. Examination of the genital tract showed a smally flabby uterus with no marked deviations from the normal. Although the bleeding had continued more or less uninterruptedly, except for exacerbations corresponding with very irregularly spaced menstrual periods, there was no marked anemia.

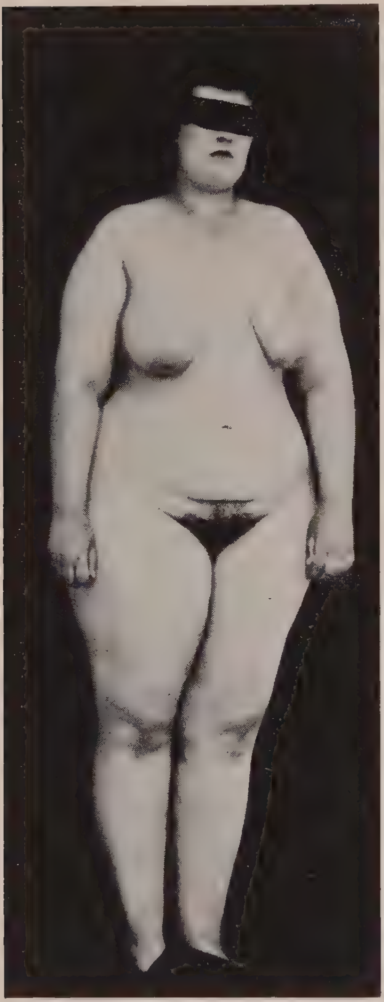


Fig. 76. Menorrhagia and Obesity.

Girl 17 years of age, weight 240 pounds, continuous bleeding. This is a rare combination.

Repeated examination of the circulating blood always showed a very low or entirely negative hormone level. I therefore classified this patient among the hypoplasias. A "stimulating" dose of X-ray was given to the ovaries. This was followed by marked improvement until finally within a few months, the menstrual cycles had become normal and this patient, to our surprise, conceived and, unfortunately, miscarried at the 5th month, of twins.

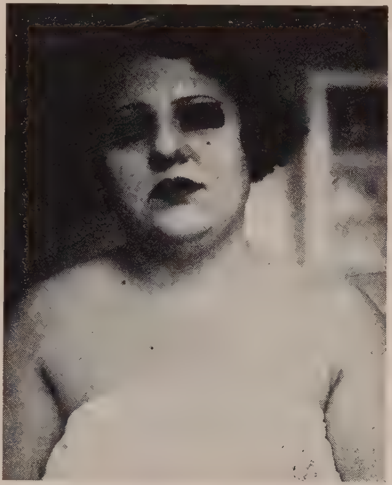


FIG. 77. OBESITY, ACROMEGALOID, HIRSUTES, METRORRHAGIA.
Bleeding for 5 years. Rare hypofunctional metrorrhagia.

A fourth patient, a young woman of 24, has bled with more or less persistence since the age of 14. Because of the puberty bleeding, a prominent colleague inserted radium in the uterus when the patient was 14 years of age, followed by a very slight and only temporary improvement. This patient shows infantilistic characters in many ways, has a poor nervous heredity, and shows a great psychical unrest. Her frame is normal. Her secondary sex characters are likewise normal. The uterus is moderate in size; the fornices poorly developed; the portio likewise. Blood examination showed a marked anemia, the hemoglobin being 46 per cent, the blood picture as follows: R.B.C. 3,000,000; W.B.C. 3,300; coagulation time 10 minutes; bleeding time 8 minutes; blood platelets 160,000; tourniquet test negative, clot retraction normal; sedimentation time 47 minutes; lymphocytes 27 per cent.

As every medical measure had been tried in vain, and several curettages had proved futile, I resorted to blood transfusion in order to improve the condition. This palliative measure has been followed by a gratifying permanent improvement in the blood picture. The bleeding has likewise diminished, but persists to a minor degree without affecting the constituents of the blood. I propose to repeat the transfusion at a later date with the hope that this functional hypoplasia will be permanently relieved. Four months after the transfusion the hemoglobin is 80 per cent, the R.B.C. 4,340,000; the W.B.C. 6,400; lymphocytes still high, 35 per cent.

Schickele and Keller (523, 524) in 1912 already expressed the view that many cases of menorrhagia and metrorrhagia were due to inactivity of the ovaries. However, they were unable to adduce any proof to their contention.

CHAPTER XI

HYPERFUNCTION OF THE OVARIES

A DECISIVE criterion in diagnosing hyperfunction of the ovaries is the demonstration of an excess amount of female sex hormone in the circulating blood. This criterion, however, cannot be applied to every case as will become apparent. Forty c.c. of blood from the circulation should contain a liberal mouse unit if taken from 3 to 5 days before the expected period. Failure to obtain this reaction in 40 c.c. of blood bespeaks a diminished production or a low threshold (see page 191). On the other hand, if 20 or 30 c.c. of blood at this time, and even more so at other times of the cycle, give a mouse reaction, an excessive amount of hormone is being secreted into the circulation or retained there.

The mere fact that a patient has a regular menstrual cycle is conclusive evidence of ovarian activity. The opposite, on the other hand, does not hold good, because, as we have shown in the discussion of amenorrhea, many patients who fail to menstruate, even over long periods of time, may have a regular blood cycle at a sub-threshold level. Patients with continuous or irregular bleeding may either show no cycle in the blood or may, irrespective of the bleeding, have a regular cycle. This fact may signify that there is an entirely different mechanism for normal and abnormal uterine bleeding.

Exaggeration of Normal Premenstrual Phenomena

General Symptoms

Normal women may note an increased feeling of pelvic fullness, mucoid vaginal discharge, slight increase in frequency of urination, and a general feeling of tension, irritability or lassitude a number of days before the anticipated menses. In many patients fullness and tingling of the breasts is observed. In a few, a slight colostrum-like secretion is noted.

Exaggeration of Breast Signs

Patients are observed in whom the premenstrual breast phenomena assume exaggerated proportions. The breasts become enlarged, tender to the touch, painful and throbby before the menstruation. Instead of deturgescing after the menstrual flow has occurred, the annoying symptoms continue undiminished. This trouble is unassociated with cystic or adenomatous breast changes. It is found in both young and old patients. Observation has convinced me that after persisting for 2 or 3 months, in spite of any treatment, the symptoms gradually disappear. No blood observations in these patients have as yet been made.

It is worth while to remember that many cases of amenorrhea show breast symptoms at fairly cyclical intervals, thus indicating the best time at which blood should be abstracted for investigating a possible sub-threshold premenstrual, or more accurately expressed, cyclical rise.

Vasomotor and Vascular Symptoms

One of the commonest symptoms which have wrongly been associated exclusively with the menopause, are flushes. These are found in hypersensitive women of all ages. This vascular instability is associated with autonomic imbalance and is by no means limited to "sympatheticotonic" individuals.

Of equal frequency are headaches and migraines which may be closely associated with the impending menstrual period.

Angioneurotic edema of the eyelids, the cheeks and extremities is not an uncommon premenstrual phenomenon. It is apt to occur at irregular intervals in the same individual but always before the period. These individuals are subject to urticaria which may be diffuse or localized. Mottling of the skin, pallor, and cyanosis of the extremities, indistinguishable from erythromelalgia and acrocyanosis may occur periodically.

Herpes simplex, and in one instance herpes necroticans, may be observed premenstrually on the lips, the labia, and occasionally in other portions of the body.

Of more serious import are the subcutaneous hemorrhages which occur by preference on the flexor surfaces of the thighs, arms, and forearms, and are rather deep-seated in most instances. Whether the severe abdominal pain complained of by some of these individuals, at this time, is due to hemorrhages into the mesentery or peritoneal coating of the intestines, I am unable to affirm. More rarely, large, widely distributed premenstrual subcutaneous hemorrhages take place, as shown in the accompanying figure. Nosebleeds, hemoptyses, and gastric hemorrhages have been reported.

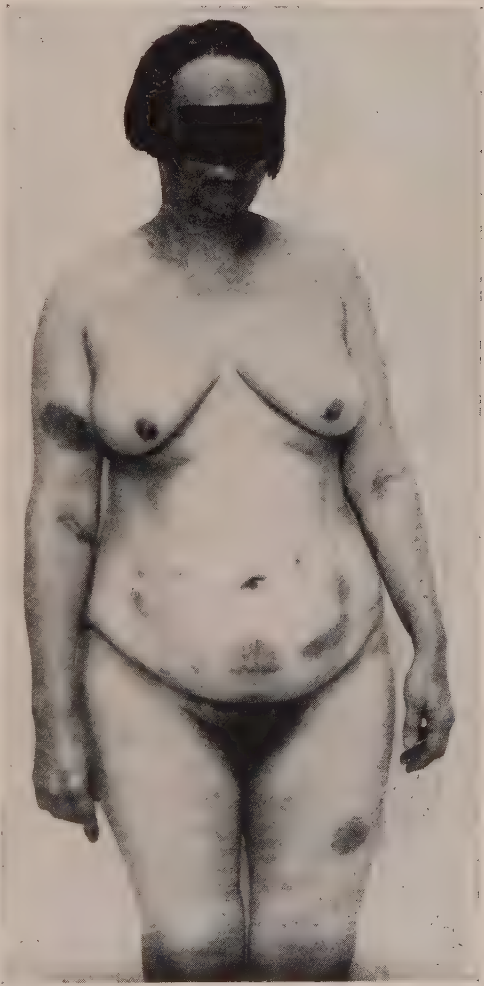


Fig. 78. Premenstrual Subcutaneous Hemorrhages.

The premenstrual pain complained of in the ovarian region has never been analyzed or fully explained (Ovarialgia). The fact that it occurs as much, if not more, in patients unquestionably of the hypo-ovarian type, inclines me to doubt whether differences in intensity of ovarian action influence or cause this pain, except in the class next to be discussed.

Excessive Premenstrual Tension

Although a considerable degree of irritability, lassitude, and increase in emotion is physiological, certain individuals are afflicted with a pathological exaggeration of these symptoms. These individuals suffer keenly from their uncontrollable irritation or depression which manifests itself in manic or melancholic crises. Sometimes the patient herself, at other times the distressed relatives, seek advice. It is well to know that the number of suicides are largest among women at the time of the approaching period.

Study of these cases has in several instances shown that an abnormal amount of female sex hormone is accumulated in the blood premenstrually.

In case 1151, B.H.

40 c.c. of blood 4 days before the menstruation = 4
30 c.c. of blood = 3
20 c.c. of blood = 0

In this instance the symptoms were of such gravity that a full castration dose of X-ray was applied to the ovaries. In spite of the proper dosage, which was given by an experienced radiologist, menstruation ceased for only 1 month, but 30 c.c. of blood taken before the next menses, did not contain a M.U. This patient has since then menstruated regularly without a return of the distressing symptoms. She has been observed for three years.

Convulsive Seizures

Convulsive seizures occurred in one colored patient in whom likewise there was an abnormal accumulation of hormone in the blood premenstrually. Complete X-ray amenorrhea was followed by permanent relief until, out of a clear sky, a new convulsive seizure, 1½ years after the induction of amenorrhea, occurred. This recurrence was fully explained within a few days by the reappearance of the menses necessitating a renewed dosage of X-ray.

The not infrequently observed hysterical "convulsions" accompanied by crying, writhing, etc. are not included in this group. They may be grouped with the premenstrual tensions. Such was No. 1318 in which four days before the period the vein blood was 3+. Abstraction of blood gave transient relief.

The question as to whether an actual oversecretion of the hormone takes place or whether these individuals have an unduly high renal and intestinal threshold, requires further study.

PRECOCIOUS MENSTRUATION

Precocious menstruation has been observed from the first month of life on in certain individuals, (See Lenz, 279; Reuben and Manning, 387), without any explanation for this startling phenomenon. In a number of instances ovarian tumors have accounted for the premature puberty and the precocious menstruation. The best known cases are those of Harris (502a), Crowell (495a), and Keatinge (507).

Binz (46), in 1925 submitted such an ovarian tumor in a child to lipoid extraction and with this extract induced premature sexual maturity in immature mice. We (Frank, Gustavson, and Goldberger, unpublished) have extracted an ovarian sarcoma obtained from a child 3 years of age placed at our disposal by Eastlake and Ingraham of Denver (the case is still unpublished). This extract produced a positive reaction in a castrated mouse.

Evidently the tubular tract of the immature child is able to respond to the ovarian stimulus as well as is that of the fetus and the adult. How much of this pathological stimulus is due to anterior lobe activity cannot as yet be determined. Both in the case of Harris and Crowell, removal of the ovarian tumor was followed by cessation of all menstrual phenomena. In due course of time, both of these children underwent the symptoms of normal puberty.

Precocious sexual maturity occurs in children afflicted with pineal and adrenal neoplasms. The pineal growths are almost uniformly teratomatous in nature. (Marburg, 514). The adrenal tumors are usually of the hypernephroma or Grawitz type. (Bulloch, W. and Sequeira, 493; Glynn, 502.) The secondary sex characters, including breast development, may be well marked. Menstruation usually fails to appear.

EXCESSIVE MENSTRUATION

If the menstrual regularity remains, but the flow is excessive, menstruation is called menorrhagia. If the cyclical regularity is obscured by the continuous bleeding, the resulting hemorrhage is called metrorrhagia. Combinations of the two are observed. The excessive bleeding observed at the time of puberty and at the preclimacterium resemble each other closely. A somewhat different type of bleeding is noted during sexual maturity.

Puberty Bleeding

With the onset of puberty, or sometimes not until the first 3 to 5 years after the menses have set in have passed, in young, usually virginal individuals, menorrhagia and metrorrhagia may prove a distressing symptom. Some of these individuals show the stigmata of infantilism, but the majority are normally feminine.

The 6 cases studied by me ranged from 11 to 17 years in age. In all prolonged, and at times irregular bleeding was noted. Curettage regularly showed cystic hyperplastic endometrium just as is encountered in the preclimacteric bleedings. One especially intractable case showed "necrosis" of the endometrium, in the first curettage, but a cystic hyperplastic endometrium in the second curettage. Several of these patients had a basal metabolism of from 10 to 15 per cent minus. The blood picture, except for the secondary anemia due to continued hemorrhage, leucopenia and occasionally lymphocytosis was normal. Both bleeding and coagulation time were regularly normal. Our test demonstrates that although the venous blood regularly showed absence of a M.U. in 40 c.c., the uterine blood, on the contrary, in some instances over considerable periods of time, contained a demonstrable amount of female sex hormone, thus differing strikingly from the uterine blood obtained in adult menorrhagias and metrorrhagias. This probably signifies a high degree of ovarian activity.

In one patient, the urine contained an unusually large amount of hormone which opens up the question whether the ovarian overfunction is even greater than appears from examination of the blood and is combined with some derangement in the excretion of the hormone.

Nos. 1076 and 1344, G. T., 14 years; began at 12 years, irregular. One year ago bled continuously for one month. Now bleeding seven weeks. Slight general infantilism; uterus normal. Hemoglobin 40 per cent; bleeding time 4 minutes, tourniquet test normal; basal metabolism normal. March 20, 1926. Vein blood negative. Uterine blood = 4.

Citrate transfusion. March 28, 1927. 12 c.c. uterine blood = 0.

Nos. 1253 and 1302, M. B., 11½ years; well developed, big breasts. Began to menstruate September, 1926 and has bled continuously since November 30, 1926 (three months). Anemic, basal metabolism normal. Uterus normal. Vein blood, November 30, 1926, as well as February 11, 1927, gave a weak positive reaction—2+.

No. 1509, O. W., 18 years, began at 14 years, regular every 28 days, for 6 days until six months ago, since which time she has bled continuously but with exacerbations. Spaced, infantile teeth, hips narrow, otherwise normal. Hemoglobin 76 per cent, coagulation time 8 minutes, bleeding time 4 minutes, lymphocytes 38 per cent. Sugar tolerance normal. Curetted August 4, 1927 (necrotic endometrium).

December 12, 1927—Vein blood = 0
10 c.c. uterine blood = 4
5 c.c. uterine blood = 4

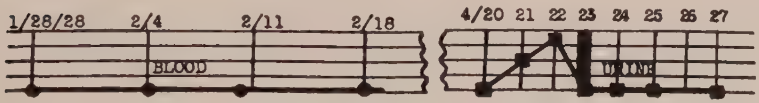
Curetted January 16, 1928 for continued bleeding. Eventually treated with X-ray.

No. 1511, M. K., 13 years, began at 11 years, every 28 days, seven days profuse. Has bled for the last 15 weeks. Compensated congenital interventricular septal defect. Hirsutes. Hemoglobin 60 per cent; lymphocytes 35 per cent; basal metabolism −10 per cent; sugar tolerance normal.

December 12, 1927—15 c.c. uterine blood = 3
“ 13 “ —20 c.c. uterine blood = 4
“ 13 “ —vein blood = 0
“ 17 “ — 7 c.c. uterine blood = 0
“ 28 “ — 6 c.c. uterine blood = 2+
“ 29 “ — 5 c.c. uterine blood = 2+
January 20, 1928 9 c.c. uterine blood = 0

Curettage showed cystic endometrial hyperplasia.

No. 1582, D. C., 15 years, began at 11 years, every 30 days for 7 days; since one year bleeds every 14 days for 10 days. Slightly overweight; hemoglobin 90 per cent; basal metabolism −11 per cent. Sugar tolerance normal. Still periodic in bleeding but excessive.



Graph 1582

No. 1760, B. K., 16 years, overweight, onset of menses at 13 years, always 2–4 weekly for 9–12 days; bleeding continuously for last 8 months; basal metabolism −5 per cent; sugar tolerance increased; sellar X-ray normal. Curettage hyperplastic endometrium. Transfusion required as hemoglobin fell to 36 per cent. Venous blood repeatedly = 0; urine repeatedly showed increased excretion of the hormone.

The work up of another case, R. M., age 19 years, has just been started. The onset of the menses dates from the 16th year, was every 28 days, 4–5 days duration. Irregular since 2 years, now every 7–21 days for 10 days or more. She has had her ovaries resected at another hospital and was later curetted, both interventions followed by no relief.

Preclimacteric Hemorrhages

Women approaching the menopause suffer from the same type of bleeding that is noticed at puberty. I refer to those women who suffer from menorrhagia and metrorrhagia without the presence of fibroids or fibrosis uteri, and in whom no demonstrable lesion is noted. Curetting, which at this age is performed as a routine measure to exclude possible intra-uterine malignancy, regularly shows the same cystic hyperplasia of the endometrium that is found in the puberty cases. In the few patients available for study, the venous blood was negative, the uterine blood either repeatedly or at times positive. At this age no hesitation need be felt to induce artificial menopause by means of the X-ray.

Menorrhagia and Metrorrhagia of the Sexually Mature

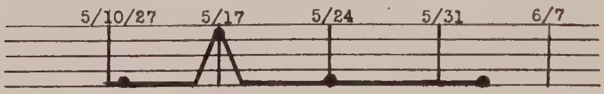
Of the 77 cases of pathological bleeding which were studied, 45 corresponded to this grouping.

The patients belonging to this group differed materially from those suffering from amenorrheas. As a group they were extremely fertile. Moreover, very few showed even slight endocrine stigmata, the great majority being normally feminine in every way.

Vein blood. When studied during the periods of bleeding, the venous hormone blood level proved uniformly low except in those cases in which cyclical exacerbation of the bleeding occurred, even while the hemorrhage was continuous. In this small group, 2 cases showed a 4 reaction after 24 and 11 days of bleeding, and one case showed a 3 upon the 3rd day of the bleeding.

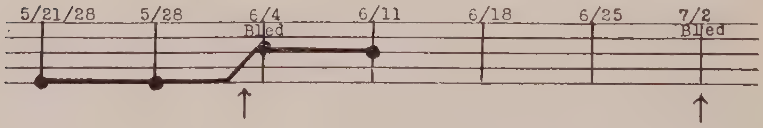
The following history illustrates the presence of a blood cycle in a patient bleeding continuously throughout the weeks she was studied.

> No. 1388, R. B., 35 years old, 1 child, 10 years ago. Menstruation always irregular and delayed until 4 years ago. Three years ago curetted and X-ray treatment of ovaries followed by only two months of amenorrhea. Bled April 17th–May 6th, 1927. Again started bleeding May 10th.



Graph 1388

> A still more atypical case was that of No. 1675, G. R., aged 27 years, married 3 years, 0 para. This patient has spotted ever since her marriage. The longest clean period has been 10 days. She was curetted 1 year ago and again 5 months ago with no relief. The patient generally and locally is feminine. In her case a protracted high level of hormone in the blood was noted while the bleeding continued.



Graph 1675

This may be a case of persistent and polycystic follicles.

Uterine blood, on the other hand, irrespective of the quantities collected, which varied from 2 to 28 c.c., was uniformly negative unless the time coincided with the menstrual period. Of the 24 bloods examined, 6 produced some reaction (between 2 and 4) all of these corresponding more or less with the 30th day of bleeding. This fact, namely the absence of hormone in the shed blood, contrasts quite sharply with the puberty group in whom a positive hormone content is rather the rule.

It will be necessary in the near future for us to study the urines of these patients in order to determine whether the urine level of excretion is normal or not. Aschheim and Zondek (24), have noted as much as 300 M.U.L. in the urine of such patients.

We know so little of the mechanism of uterine bleeding that it would be hazardous to express even an hypothesis based on these comparatively few findings. The appended chart of menorrhagias and metrorrhagias shows that the vein blood throughout the days of bleeding, appears to be at a low level.

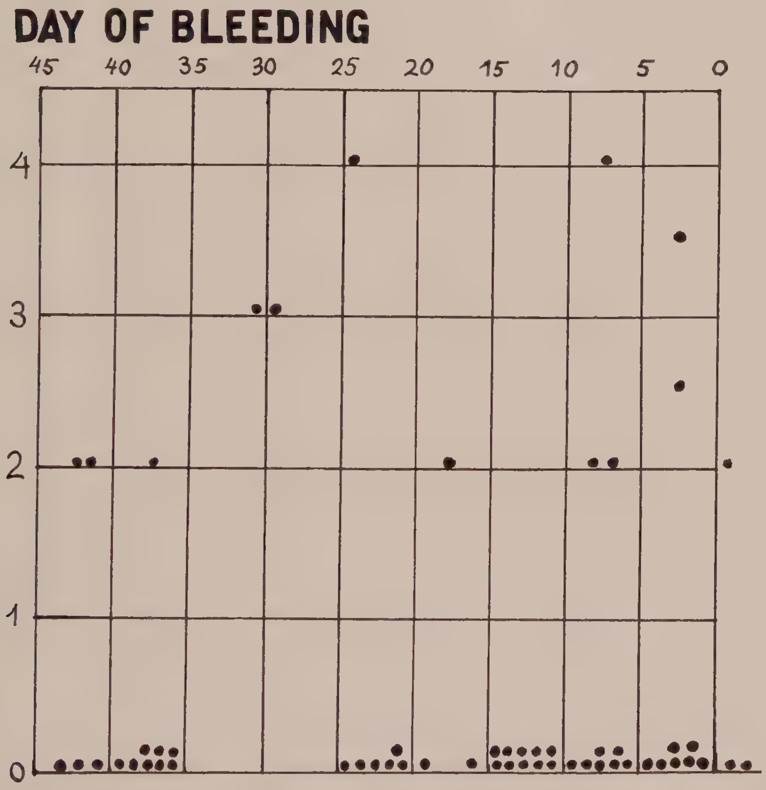


Fig. 79. Chart of Menorrhagias and Metrorrhagias.

Showing the amount of female sex hormone in the circulating blood after various days of bleeding.

The pathology underlying these functional bleedings is extremely misleading. No explanation based upon anatomical findings has stood the test of critique. The proof that such a condition *may*, even if it must not be based upon purely functional endocrine influences, is shown by the next group of cases.

Menorrhagia and Metrorrhagia Due to Endocrine Disturbances

Just as the gynecologist sees patients with amenorrhea whose trouble is due to some general disease such as tuberculosis, or some climatic disturbance such as immigration from Ireland to the United States, or as the result of myxedema due to thyroid inactivity, so the gynecologist is also confronted with cases of menorrhagia and metrorrhagia which appear during some stage of an endocrine disease. Menorrhagias are most often seen at the beginning of hyperthyroidism (whether this be of the Basedow or toxic type), at the onset of acromegaly, or other hyperactivity of the pituitary. This stage is commonly followed by amenorrhea as the primary endocrine disease advances. A good instance is shown by the following case.

Mrs. E. S., 32 years of age, married 1½ years, no pregnancies. This patient was regular until June, 1928 when she skipped 2 periods. She then bled continually between September 24th and November 1st and again from December 1st, 1928 to February 7th, 1929, when seen by me.

Examination showed a patient with normal sized thyroid gland, a slow, regular, normal heart action, no endocrine stigmata, except for a somewhat infantile pelvis. The uterus was cochleate, the cervix hypoplastic, the adnexa normal. There was a moderate amount of bleeding; hemoglobin 76 per cent, red blood cells, 4,400,000; leucocytes, 4,400 of which 32 per cent were lymphocytes. The basal metabolism on February 9th was +61 per cent, on March 6th +56 per cent.

This patient, although she showed none of the usual symptoms of hyperthyroidism, was suffering from a very greatly increased basal metabolism for which she is being treated, and upon the reduction of which the menorrhagia and metrorrhagia should diminish.

More rarely I have observed menorrhagia and metrorrhagia at the onset of acromegaly. Even less frequent than this cause is the bleeding due to diminished thyroid function.

In the Froehlich type of pituitary disturbance, adrenal underfunction and diabetes I have regularly noted amenorrhea from the very beginning of the disease.

Fibromyoma Uteri

This group, like the preceding menorrhagia group, appears extremely fertile. Only 12 uncomplicated fibromyomata were available for this study. Of these, 5 preserved regularity of bleeding (menorrhagia), the other 7 having lost all semblance of periodicity, for the time being, their bleeding having existed for from 8 to 16 weeks. Three of the 12 patients were nulliparous, the other 9 had had 25 children, the greatest number for one patient being 6. The vein bloods in all of the cases were negative. Of the uterine bloods, only 3 showed a positive reaction.

The question has frequently been posed whether a continued persistence, that is a high hormone level in the blood, accounts for the presence of fibroids. As is well known, after castration, atrophy of the tumors usually takes place. It is quite conceivable and in fact an attractive theory, that the causation of fibroids is due to the influence of the female sex hormone which for some, as yet unexplained reason, causes an extremely localized stimulus of the muscle fibres. That in contrast to this a pathological stimulus of the entire uterus can be produced, has become evident. See next group (fibrosis).

After the fibroids have sprung into existence and attained some size, the new vascular conditions produced, as well as the lymphstasis, then appear to play as great a rôle as the stimulus exerted by the female sex hormone.

Fibrosis Uteri

Unfortunately the cases of fibrosis uteri could not be studied independently from the cases of menorrhagia and metrorrhagia. The condition occurs in the preclimacteric, as well as in younger parous women, who complain of hemorrhage, sometimes dull pelvic pain, and an increase of discharge. In the early stage, a symmetrically enlarged, boggy, hyperemic uterus is found with increase of bleeding. At a later stage, the uterus is found uniformly enlarged, symmetrical, hard. Examination of such a uterus shows marked thickness of the muscular layer in which an overgrowth of fibrous tissue is noted. The arteries project as pipe stem tubes. The ovaries may look normal or polycystic and enlarged. However, the condition must be classified as due to strictly functional causes and is in every way comparable with fibromyoma except that the stimulus has produced a diffuse hyperplasia which affects the connective tissues more than the muscle fibres.

Inflammation of the Ovaries

It is quite impossible to dissociate all bleeding conditions in which inflammation plays some rôle from the purely functional conditions, unless the anatomical findings are controlled by operation or autopsy. In recognizably inflammatory disease the menstrual cycle may be unaffected, the bleeding may be increased in amount and frequency, or, very rarely amenorrhea may supervene. The inflammatory adhesions favor increased atresia and polycystic ovarian changes. It is surprising to note how normal the cycle may continue in the presence of severe, acute tubo-ovarian inflammation. No blood studies have as yet been undertaken.

Dysmenorrhea Membranacia

In contradistinction to the simple functional dysmenorrhea which is usually a symptom of hypoplasia, I am forced to classify dysmenorrhea membranacia, on the strength of the single striking case at my disposal in the class of hyperfunctional conditions.

The patient L. H., No. 1292, 36 years of age, married, never pregnant, passes a cast with each period. This cast consists of the functional layers of the endometrium (see p. 36). There is occasional muco-membranous colitis. No family history of similar condition could be obtained.

The vein blood taken 3 days before the expected period showed +3. Vein blood taken 6 hours after the period had started was still 3. Ten c.c. of menstrual blood during the first day showed 2+. These findings would signify an unusually high level in both circulating and menstrual blood and might account for a considerable overproduction of pregravid endometrial changes with consequent more massive expulsion of menstrual decidua.

As previously mentioned, I am inclined to place the functional dysmenorrheas in the class of hypofunctions. The dysmenorrheas occurring after childbirth are usually of inflammatory etiology and are due to the monthly lighting up of subacute or chronic inflammations of the adnexa in consequence of the premenstrual turgescence.

DYSMENORRHEA WITH TENSION

Occasionally dysmenorrhea occurs accompanied by marked premenstrual tension. In these cases the dysmenorrhea is acquired. Such an instance is case No. 1316 in which a patient, 40 years of age, complained of increasing dysmenorrhea. The local findings proved negative. Four days before menstruation, the vein blood was 3. With the flow, all symptoms abruptly disappeared.

CYANOSIS: VIRILISM

In a number of women of masculine type, often accompanied by hirsutes, bass voice, assertiveness, and an approach to masculine force and assurance, I have noticed a cyanosis of the genital tract showing itself in violaceous tinge of the vulva and cervix. These women are often sterile but possess an unusual degree of libido, sometimes verging toward nymphomania. None of the bloods of these patients have been studied.

OVERSEXED

In the lower animals the desire to copulate is limited in the female to the time of estrus, with the few exceptions previously referred to. (See p. 106). This is definitely demonstrated by the fact that in castrates the desire to copulate may be re-established by the induction of artificial estrus (6).

In the primate series, among the higher apes and in the human, sexual relations are indulged in throughout the menstrual cycle. In a limited number of women, libido is distinctly increased at the premenstrual time. However, a great independence between libido and ovarian function is noticeable in the human female. Numerous observations, carefully controlled, have fully convinced me that women, after double oöphorectomy, undergo no diminution in their sex desire. The same applies to those who have passed through the normal menopause.

That the establishment of libido can occur independently of the ovaries is further evidenced by the following case.

A young woman at the age of 20 was subjected to double oöphorectomy because of bilateral carcinoma of the ovaries. She was married the following year at the age of 21. Now 6 years have elapsed and on inquiry, she declares that her sex desire is normal, well developed, and culminates in typical orgasm. This patient had had neither sex relations nor any well developed inclination toward men before her operation.

On the other hand, among the human race, real cases of nymphomania in which the sex desire is pathologically increased, are not benefited by double oöphorectomy. According to veterinarians, in nymphomanic cows, in which the ovaries are usually found to be polycystic, castration sometimes produces complete relief of symptoms. Frei and Lutz (500).

In two instances a marked increase of libido was complained of by patients who had long since passed the menopause.

No. 1601, B.L., aged 60 years, who had had 10 children, had entered the menopause 6 years previously. For the last 3 weeks the patient has had a feeling of fulness in the vagina and pelvis, accompanied by an undue and constant sex desire. Examination showed an atrophic vulva and vagina, slight rectocele and cystocele. The uterus and adnexa were not enlarged. Blood taken on February 16th, 1928, as well as February 23rd, 1928, in both instances showed a well-marked, although weak positive reaction +2.

No. 1651, age 62 years, 4 para, has never entirely lost her period, which, however, during the last few years, has became somewhat irregular. For this reason, 50 X-ray treatments were given. No details could be obtained. Radium was also inserted in the uterus. This patient weighed 219 pounds, was difficult to examine, but showed no senile changes in the vulva or uterus. Forty c.c. of vein blood, April 17th, 1928, showed a +3. On April 24th, 1928, 25 c.c. gave a +2, which is a very weak reaction for 40 c.c. but strong for this smaller amount. This patient was watched and later a diagnostic curettage was performed by her physician. The curettings showed a beginning adenocarcinoma of the fundus. It is tempting to try to ascribe the origin of adenocarcinoma uteri to such an undue and protracted hormonal stimulus.

Post Climacteric Hemorrhages

Post climacteric hemorrhages with one possible exception, are practically always mechanical in origin. Among such etiology I include the bleeding from fundal or cervical carcinoma or from other intra-uterine neoplasms in which the hemorrhage is caused by erosion of vessels or sloughing of tissues with resultant ulceration. Whether the bleeding which accompanies twisting of the pedicle of an ovarian cyst is strictly mechanical or whether the prolonged hyperemia of the previously quiescent ovary stimulates a new formation of female sex hormone, I am as yet unable to say.

Real regeneration with temporary reawakening of ovarian function has now been produced in senile animals by means of anterior pituitary lobe extracts. See page. 19.

VICARIOUS MENSTRUATION.

I have never been fully convinced of the similarity between vicarious bleeding and menstrual bleeding. It will be remembered that premenstrually subcutaneous hemorrhages are very common, but these should in no way be compared with the menstrual flow. The only case in which any examination of a patient with "vicarious menstruation" could be made was case No. 1327, patient, age 43 years, had had a supravaginal hysterectomy and supposedly double salpingo-oöphorectomy two years previously. For one and one-half years after operation no symptoms were noted. From then on, regular monthly bleeding took place from the left nostril. Our own records show that the bleeding occurred March 16th, 1927, April 16th, 1927, June 14th, 1927, the report of bleeding in the intermediate month also being received but not personally controlled. Vein blood taken at the time of bleeding on March 16th and April 16th was negative. Eight c.c. of blood obtained from the nostril on June 14th was likewise negative.

It is of course possible that some regeneration of a fragment of ovary, left behind at the time of operation, could have taken place but the case is far from conclusive.

THE MENOPAUSE

Only few bloods of patients who had passed the menopause were tested. Usually these patients presented themselves for examination because of the fear of pregnancy. It was necessary to observe the patient for a considerable period to exclude amenorrhea of other origin.

No. 1193, 44 years of age, amenorrhea for 6 months
50 c.c. vein blood = 0.

No. 1251, 45 years, amenorrhea for 8 months.
40 c.c. vein blood = 0.

CHAPTER XII

PREGNANCY DIAGNOSIS: DIFFERENTIAL DIAGNOSIS

THE serum of pregnant women does not contain a sufficient amount of female sex hormone to be utilizable as a test for pregnancy, although this was attempted by Binz (46), Trivino (443) and Fels (120). For example, in Experiment 1375, I found that

16 c.c. of pregnancy blood serum gave 4
18 c.c. of pregnancy blood serum gave 4
20 c.c. of pregnancy blood serum gave 4.

On the other hand, in Experiment 1352, a series of sera gave the following results:

6 c.c.—0
7 c.c.—0
8 c.c.—0
11 c.c.—0
14 c.c.—0

The extract of 40 c. c. of vein blood gave a 4. It will thus be seen that as much as 15 c. c. of serum are needed to give a definite reaction. It has been found impossible to inject such large quantities into test mice and therefore we have not utilized this test.

A gradual increase in amount of female sex hormone present in the blood is noticed from the 8th week of pregnancy on to term. It is unfortunate that there is a negative phase between the time of menstruation and about the 7th week. This greatly impairs the usefulness of the test, as an early pregnancy test is most to be desired. The following table shows the increasing reliability of the test after the 8th week has been passed.

165 Bloods from 150 Pregnant Women

	1–4 WEEKS	5–8 WEEKS	9–16 WEEKS	4–6 MONTHS	6–8 MONTHS	8–10 MONTHS
	per cent	per cent	per cent	per cent	per cent	per cent
Positive	5	73.5	84	85	95	97
Negative	95	26.5	16	15	5	3
Total Bloods	20	45	25	20	21	34

Total negatives 23.5 percent; after 8th week 5.5 per cent (Fig. 55, p. 194, shows the above in graphic form)

After labor a sudden decline in the hormone level of the circulating blood takes place. The same applies to the amount of female sex hormone excreted in the urine.

We have been unable to utilize the Aschheim and Zondek test for detection of early pregnancy (24) (see p. 19) because, in the first place a larger mouse colony must be kept on hand than we are able to accommodate in order to supply the immature mice required for the test. In the second place, our repeated attempts to follow their technic have invariably resulted in the death of the immature test mice long before readings could be obtained. We have not had the opportunity to test out the "Louria" (512) and Mazer and Hoffman technic (330). Their percentage of errors seems to be even greater than our own.

> Since the completion of this manuscript, in response to a personal communication of Prof. Aschheim, we have repeatedly tried the pregnancy test. The immature mice were obtained through a dealer and with this larger stock on hand, we were able to perform the test with considerable success although the mortality is large. The blood points in the ovaries are unmistakable, and a positive reaction is recognized. This is by far the best test for pregnancy as yet discovered.

Interpretation of the Female Sex Hormone Reaction

In interpreting our test, it must be remembered that a +3 or even a 3+ obtained from vein blood in the early weeks of amenorrhea may signify impending menstruation. Therefore, it is always necessary to combine the clinical with the serological findings. To illustrate:

In Case 1258, amenorrhea existed for 2 months. The vein blood showed +3, and the patient had a normal period the next day.

In Case 1165, amenorrhea had existed for 5 months. The vein blood showed a +3. The patient bled the same day.

If any organotherapeutic medication had been given to these patients, the report of a miraculous result of the therapy would have appeared in the literature as has so often happened in the past.

Tests, if taken too early, will uniformly prove negative as during the 4th week we obtained only 5 per cent of positive results. Under such condition, repetition of the test should be made, as is shown by the following case in which the diagnosis of pregnancy was made.

No. 1394—"Irregular menses"—every 2–3 months. 29 years; 2 para; hyperthyroidism.
Last menstruation—March 28th, 1927.
Blood taken
May 13th, 1927 = 0 (six and one-half weeks)
May 27th, 1927 = 4 (eight and one-half weeks)
Later aborted at 6th month.

DIAGNOSIS OF PREGNANCY

The importance and the value of our test in diagnosis is best illustrated by detailing a number of cases.

Amenorrhea vs. Pregnancy in the Obese

In these patients pelvic examination is ordinarily misleading or impossible. Therefore in these cases, particularly, the test is of great value.

No. 1455. Obesity; amenorrhea; pregnancy? 25 years; 1 para; previously regular menses; weight 239 lbs.; feels she is pregnant.
Last menstruation—April 4th, 1927.
Blood taken October 17th = 0
October 24th = 0
October 31st = 0.
Diagnosis: Pseudocyesis. Reduced to 198 pounds by diet and thyroid extract. Blood taken January 9th, 1928 = 2+. Menstruated January 15th, 1928.

No. 1581. Impending Menopause? Pregnancy? Spotting. 39 years; 9 para; flushes, dizziness, very obese.
Last Menstruation—October 23rd, 1927.
Blood taken January 25th, 1928 = 3+ Surely pregnant.
March 29th, 1928 = 3+ Feels life.

In both of the above obese patients, diagnosis at this stage of gestation without the test would have been impossible.

Pregnancy vs. Fibromyomata

Gynecologists and obstetricians are often confronted by the quandary arising from the presence of soft fibroids which may be mistaken for a gravid uterus, or pregnancy and coincidental occurrence of small fibroids. Here our test has frequently proved of value.

No. 1420. Is a simple case; 32 years; 1 para; fibroids size of three and one-half to four months' pregnancy, vomiting.
Last Menstruation June 2nd, 1927.
Blood taken June 30th, 1927 = 0.
Certainly not 3 months pregnant. Found not pregnant. Test taken too early for a final answer.

No. 1457. Woman 38 years; 10 para; short, obese.
Blood taken October 21st, 1927 = 0; not pregnant.

No. 1614. Patient not seen by me. Obstetric service of another hospital said "fibroids"; gynecological service said "pregnant." History of irregular bleeding. Blood received February 24th, 1928 = 4. Diagnosis. Pregnancy. She carried through.

No. 1485. "Fibroids." Denied possibility of pregnancy. 42 years; 4 para; 2 fibroids, each size of an orange.
Last menstruation September 29th, 1927.
Blood taken November 22nd, 1927 = 4.
7 weeks pregnant.

No. 1704. Patient not seen by me. Had had laparotomy performed; "pregnant" uterus found and abdomen reclosed on September 7th, 1928. Blood sent.
October 1st, 1928 = 0;
again October 10th, 1928 = 0
October 29th, 1928 = 0
Was reopened and fibroid removed.*

The above illustrative cases indicate that our reaction has proved of real value in differentiation.

Pregnancy vs. Malignant Abdominal Tumor

In the case of patients who have undergone amputation of the breast for carcinoma, pregnancy greatly favors recurrence, because of the tremendous congestion and stimulation of all breast tissue which takes place during gestation. Therefore an early diagnosis of pregnancy is of vital importance in order to induce abortion. Moreover, the question of castration must be considered in these cases. Twice within a short time, the difficulty of distinguishing between pregnancy and pelvic tumor has been simplified by the test as the following two cases will show.

1721. Patient not seen by me. 40 years of age. Six months previously amputation of breast for carcinoma. Last period 4 months ago. Has large mass in lower abdomen. Blood received November 21st, 1928 = 4.
Diagnosis pregnancy. Uterus emptied by hysterotomy.

No. 1756. Patient 30 years. Amputation of breast 2 years previously. Four months amenorrhea. Now intractable vomiting. Pelvis occupied by large soft mass. Retroflexed incarcerated pregnant uterus or metastases?
Blood taken March 11th, 1929 = 0.
Diagnosis: metastases.

Lactation Amenorrhea vs. Pregnancy

The physician is often appealed to by a worried mother who, for some reason, believes that she has conceived during lactation.

In case No. 1670, the additional factor of severe diabetes had to be considered. The patient had been delivered 5 months previously of twins and had nursed them since. She now complained of morning nausea and was consequently very much worried. Blood taken on May 14th, 1928, proved negative and the subsequent course has shown that she was not pregnant.

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* Such an error can be avoided at operation by aspiration of the uterus. In case of pregnancy, liquor amnii would be obtained. A cystic fibroid might conceivably mislead because then also fluid would be aspirated.

Menopause vs. Pregnancy

The physiological cessation of menstruation at the menopause is often preceded by irregularity of bleeding. Both amenorrhea and irregular bleeding prove disquieting to women, especially to the mothers of grown children who are far from desirous at this age to increase their family. The difficulty of diagnosis may be found increased by the fact that at this period of life the uterus is often enlarged by repeated parity and by fibrosis uteri. Not infrequently, also multiple small fibroids increase the volume of the womb. Such cases are well illustrated by No. 1581, page 249 in which there was first amenorrhea and then spotting, and where the patient was really pregnant.

Case No. 1485, page 249, is one in which the patient, who had not conceived for many years, was quite incredulous when informed, by means of our test, that she was 7 weeks pregnant.

Case No. 1563 was 41 years old and an 8 para. Her last menstruation had occurred November 29th, 1927. The blood taken January 13th, 1928 was negative but as only 6 weeks of amenorrhea existed, we considered it wiser to repeat the test 1 month later. On February 14th the result was 3+, proving that a 10 weeks pregnancy now existed.

No. 1547, 4 para, was 44 years old, her last menstruation occurring in November, 1926. The blood taken on January 4th, 1927 proved negative. The diagnosis was "not pregnant" but "menopause." This likewise proved true.

Pseudocyesis

Ludicrous and mortifying errors both to patient and physician can be avoided by means of the test. No women are harder to disabuse of preconceived ideas than women who insist upon being pregnant.

Case No. 1455 was that of a patient 25 years of age who had had one child which she lost. She was always regular until the last period which occurred in April, 1927. Her weight had increased from 190 to 239 pounds. She vomited. She believed herself pregnant. Examination did not show enlargement of the uterus but the patient refused to be undeceived. We therefore took blood on October 17th, 1927 which was negative and again on October 31st which likewise proved negative. This patient was very difficult to convince. Her weight was reduced by appropriate measures and menstruation returned.

No. 1644. This patient was 26 years of age, married 5 years, had never conceived. Menstruation was regular until August 31st, 1927. When seen in April, 1928, the patient declared herself 7 months pregnant and felt life. She had been observed and treated for her "pregnancy" for the last 5 months by her family physician. We found the uterus not enlarged and blood taken April 2nd, 1928 was negative. It was a difficult task to disillusion both the patient and her medical attendant.

Diagnosis of the Condition of Fetus and Placenta

It seemed likely that the death of the fetus, which entails the interruption of the fetal portion of the placental circulation, might have an influence upon the hormone production and hormone level in the blood. On the other hand, I was aware from experimental work preformed by me (137) that after fetal death, the placenta remained in a surprisingly good condition of nutrition as long as vascular contact with the uterus of the mother was maintained. Because of this complicated state of affairs, the result of the test is sometimes difficult or impossible to interpret correctly. However, even with this handicap, the test has proved of distinct clinical use to us in this connection.

Retained Placenta at Term

Case 1403, A. L. D. was admitted to my ward with the history of having been delivered, at a difficult labor, of a dead fetus at full term on May 9th, 1927, after a very disturbed pregnancy during which high blood pressure and albuminuria had been noted. An irreconcilable difference in the statements of patient and physician existed. The patient asserted that the afterbirth had not come away. The doctor on the other hand, declared that the placenta had been passed. The patient was admitted 17 days after labor with a uterus reaching one-half way to the umbilicus, with little lochia and no foul discharge. A blood test was taken May 26th, 1927 and gave a+3. The complete placenta was manually removed piecemeal. It was found in fresh condition, not especially adherent, but evidently in direct contact with the maternal circulation. I am glad to be able to report that in spite of this inexcusable obstetrical error, the patient made an uneventful recovery.

It is of further interest to note that by the lipoid extraction of this placenta, 10 grams were found to contain a M.U.which is about one-tenth the amount which a fully functioning placenta contains.

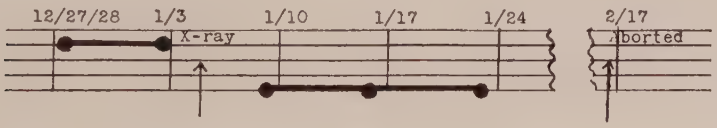
Missed Abortion

No. 1320. This patient, S. R., age 29 years, nullipara, was known to to have small fibroids before pregnancy. Regular menstruation until August 16th, 1926. Patient was seen in consultation March 9th, 1927 when there was a mass reaching to within 1 finger of the umbilicus. This impressed me like a uterus of about three and one-half to four months pregnancy with a pedunculated fibroid on its anterior surface. The menstrual history would lead one to expect a 7 months pregnancy. On March 9th, 1927 a blood test was taken and found negative. The diagnosis of death of fetus with missed abortion was made and soon verified by the spontaneous expulsion of the regressed products of conception.

Death of Fetus After X-Ray

It has been our practice for several years to produce therapeutic abortion in certain patients suffering from grave and chronic diseases, by means of the X-ray. (See Wyser and Mayer, 527). The method is restricted to patients suffering from severe chronic cardiac disease, tuberculosis, etc. in whom future pregnancies can not be countenanced.* In these cases, it is of the utmost importance to be able to determine whether the treatment has been adequate and effective because even in successful cases, a period of a month or more may elapse between the time of raying and the expulsion of the regressing products of conception. These strikingly resemble a "missed abortion." Two cases in point will illustrate this.

No. 1736, V. I., 39 years of age, a very bad cardiac, extremely obese, has not menstruated for 10 months. On December 27th, 1928, blood taken showed +3. The diagnosis of pregnancy was therefore definitely established because of the blood findings. The patient incidentally complained of nausea and vomiting but pelvic examination gave no information because of the tenseness of the recti, and the extreme obesity. The appropriate dosage of X-ray was given on January 5th. January 14th blood examination was negative. For the sake of safety a repetition of the blood test was made on the 22nd of January and again proved negative. The macerated products of conception were aborted spontaneously on February 17th.



Graph 1736

No. 1701, on the other hand, was three months pregnant, aged 45 years, with extreme hypertension and bad cardiac disease. X-ray was given, but in too small dosage. The patient was not seen for one month. Vein blood then showed +4. The fetus was alive and abortion was induced in an outside institution because of threatening symptoms.

Another of our three failures, in some sixty cases of induction of abortion by the X-ray, was controlled by the female sex hormone test.

No. 1386, severe cardiac, aged 27 years, last period January 10th, 1927, was seen when three and one-half months pregnant. X-ray was given May 6th.

Vein blood May 6th = 3
" " May 15th = 2+
" " May 27th = 3
" " June 6th = 3

Hysterotomy was performed the pregnancy having continued.

A typical case of X-ray abortion is the following.

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* Since losing a patient suffering from cardio-vascular disease and nephritis on the table, during the induction of a therapeutic abortion, I have more and more preferred the X-ray method which requires no operative intervention and eventually terminated in spontaneous. almost bloodless, abortion.

No. 1370, age 36 years; severest type of hyperthyroidism, 7 para, no response to medical measures. Last menstruation February 27th, 1927. Vein blood taken April 23rd =4. X-ray given April 26th.

Vein blood May 4th (8 days after radiation) =3
Vein blood May 25th (30 days after radiation) =0

Spontaneous abortion, June 10th.

Another such case is No. 1424, aged 29, severe mitral stenosis, last menstruation April 25th, 1927. Seen July 9th, then 8 weeks pregnant.

Vein blood July 9th =3
X-ray given July 11th.
Vein blood July 20th =0
Spontaneous abortion.

Threatened Abortion

Obscure causes of bleeding after amenorrhea are difficult to interpret. If pregnancy is suspected or diagnosed, the question as to whether the abortion is "threatened" or "inevitable" is of importance. I have known patients to be kept in bed for weeks and even months, where after the expulsion of the products, it becomes evident, from the discrepancy in size between fetus and time, that death of fetus took place weeks previously and that the precautions had simply delayed emptying of the uterus and recovery.

Threatened vs. Inevitable Abortion

No. 1380 was the patient of a colleague. Last menstruation, 4 months before. Found pregnant and very desirous of children. Has been spotting and bleeding for 4 weeks in spite of bed rest and opiates. I was asked whether it was worth while to continue these strict precautions. May 2nd, 1927 blood taken was negative. This was strong evidence in favor of death of fetus but precautions were not relaxed. One week later the uterus emptied itself spontaneously.

No. 1469, last menstruation August 30th, 1927, began staining slightly but persistently October 9th. Uterus enlarged to the size of 3 months pregnancy. Blood taken November 4th =0. Patient's physician was interrogated and reported that she aborted the day after abstraction of blood. The abortion consisted of old products.

Incompleted Abortion

In the frequent instances of incomplete abortion, commonest in the 8th to 12th week of pregnancy, in which persistent bleeding continues, the blood test is regularly negative and of no value (Cases: Nos. 1350, 1356, 1446, 1451, 1469).

EXTRA-UTERINE PREGNANCY

Only 7 cases have been examined. As the test does not become positive before the 7th or 8th week, it is of no use in early cases.

In two cases of rupture, the peritoneal blood was found positive at 45 and 50 c.c. The vaginal blood shed after the death of fetus was found negative just as post partum blood, after the first few hours, is negative (Case No. 1528, 36 hours after operation). The test may prove of use in the diagnosis of obscure abdominal pregnancy after the 8th week.

Failure of the Test

Experience has shown us that no positive reaction can be looked for before the 7th week of gestation. If this is kept in mind, errors can be avoided.

Case No. 1563 on January 13th, 1928, 6 and one-half week's amenorrhea, gave vein blood =0; on February 14th, 10 weeks =3+.

I have a wholesome dread of interpreting bloods obtained from outside sources, as it is impossible to judge whether the full quantity (40 c.c.) was sent and how long the blood has been kept. Recently two such bloods were sent by the new resident of a hospital service in which our interpretations previously had been uniformly successful. In both instances, the bloods taken on the same day proved negative although the subsequent course showed that pregnancy of more than 8 weeks existed (No. 1727, No. 1728). Almost assuredly some grave error in technic was committed.

Extraction of Human Tissues

As a matter of interest the following data are appended. All tissues were ground, extracted with alcohol and ether. The ether residue was emulsified in water and injected in a test mouse.

Fresh placenta, 3rd months;	1 gm.	=4	=1000 M.U.Kg.
Fresh placenta, 8th months;	1 gm.	=3	= 900 M.U.Kg.
Fresh placenta, term	1 gm.	=2+	= 800 M.U.Kg.
Cord blood, term	35 c.c.	=4	= 28 M.U.L.
Placenta from slow abortion;	10 gm.	=3	= 90 M.U.Kg.
Placenta from X-ray abortion;	10 gm.	=3	= 90 M.U.Kg.
Corpus luteum, early pregnancy;	2 gm.	=4	= 500 M.U.Kg.
Corpus luteum early tubal pregnancy;	2.5 gm.	=4	= 400 M.U.Kg.
Corpus luteum cyst fluid;	1 c.c.	=3+	= 950 M.U.L.
Simple ovarian cyst fluid;	1 c.c.	=4	=1000 M.U.L.
Follicle cysts, sterility;	2 c.c.	=4	= 500 M.U.L.
Parovarian cyst fluid;	5 c.c.	=0	
Papilliferous ovarian cyst fluid;	3 c.c.	=0	
Pseudomucin ovarian cyst fluid;	3 c.c.	=0	
Serous ovarian cyst fluid;	3 c.c.	=0	
Saliva of pregnant women;	30–60 c.c.	=0	
Embryo of 8 weeks;		=0	
Bile of male;	10 c.c.	=4	
Bile of male (fistula);	150 c.c.	=0	

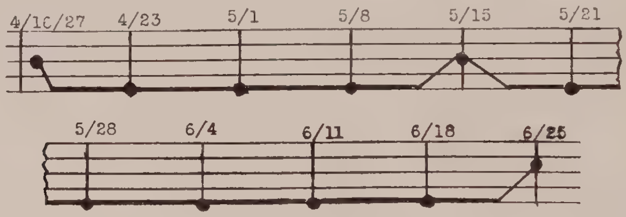
CHAPTER XIII

STUDY OF THE FEMALE SEX HORMONE IN VARIOUS CLINICAL CONDITIONS

1. Do the Ovaries Function After Hysterectomy?

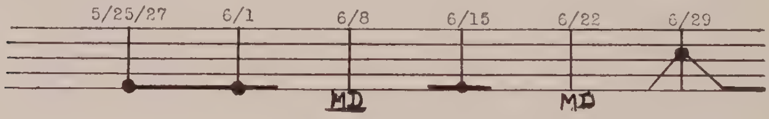
THREE cases in which one or both ovaries had been left *in situ* after removal of the uterus have been studied. They are of sufficient importance to warrant description.

No. 1363, M.V.A., 35 years of age, had had a supravaginal hysterectomy and unilateral salpingo-oöphorectomy 17 years before. She still complained of flushes and showed slight kraurotic changes on one labium. Blood examination was done over a long period of time and showed that even after the elapse of 17 years the ovary was still functioning regularly.



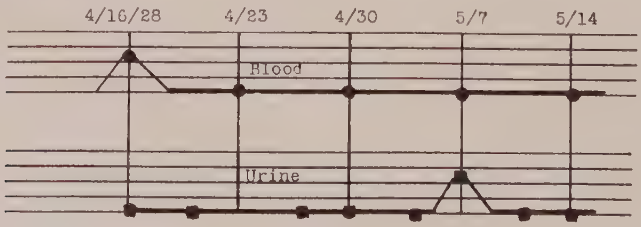
Graph 1363

No. 1402, R. L., 34 years; hysterectomy and unilateral salpingo-oöphorectomy April 4th, 1927.



Graph 1402

No. 1650, T. J., 27 years; hysterectomy, both ovaries left; operated upon January, 1928. Has flushes. Both the blood and urine showed changes in the hormone level.



Graph 1650

Even with these few cases it becomes evident that the ovaries continue to function and that the blood cycle is not abolished. It also proves that excretion takes place by other channels than by the menstrual blood. Among these channels the urine appears to be one of the important emunctories.

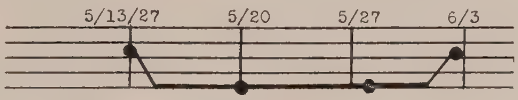
Hot flushes do not signify cessation of ovarian action.

2. Determination of Sex: Absence of Vagina

In 1926 we (Frank and Goldberger) described a series of patients who lacked vagina and probably also uterus. In these, by means of the female sex hormone test, we demonstrated a cyclical blood reaction which we interpreted as indication of feminine gender (i.e. ovarian action). This conclusion appears warranted, although a female sex hormone reaction has been obtained in the blood and urine of males. But in males no cyclically recurring reaction has been found.

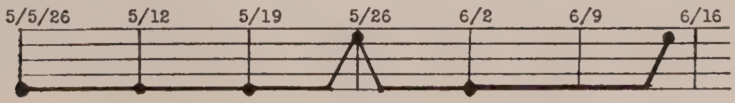
In all, ten cases were seen and of these six have been studied.

A typical case is that of No. 1393, J. K., aged 20 years, married and threatened with divorce because of impotentia coeundi. While in the hospital for construction of a vagina by means of the tube-flap method of Frank and Geist (499) a careful blood study was made.



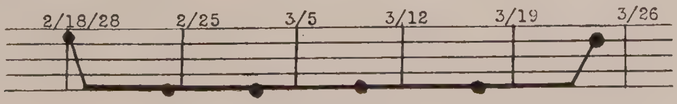
Graph 1393

No. 1399, E. S., 25 years, is another. As can be seen from her picture, she is feminine though somewhat infantile, has strong heterosexual feelings and is engaged to be married. Vulva normal, vagina and uterus absent, ovaries not felt. This patient shows a very irregular cycle in both blood and urine.



Graph 1399

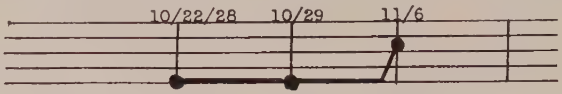
No. 1608, R. L., 21 years. Typically feminine except for absence of vagina and uterus. Has little sex feeling or inclination. Her blood shows the following:

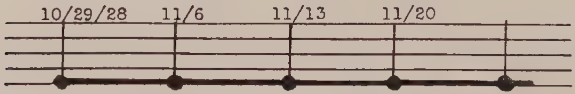


Graph 1608

Both No. 1078 and No. 1131 showed positive (+3) reactions. No. 1713, 17 years of age, rather dull and retarded, showed a positive (2+)

reaction. No. 1717, R., 20 years of age, very feminine, coquettish, bright and alert, on the other hand gave no evidence of ovarian cycle.





Graph 1713
Graph 1717

These patients possess functioning ovaries and may show a typical cyclical reaction.

In No. 1399 a laparotomy had been performed at the age of 16 years but as no specimen had been removed from the exposed gonads, no determination of sex was made at that time.

If the female sex hormone test demonstrates that cyclically accumulation of the hormone occurs in the blood, or if the hormone can be demonstrated at regular intervals in the urine, I consider this evidence of the presence of functionating ovaries and the patient a female.

3. Endocrine Cases

The typically endocrine cases seen by me showed only two deviations in their sexual tract—overfunction and underfunction. Therefore many of them have already been dealt with incidentally in Chapters X and XI.

Pituitary Conditions

The cases of Froehlich's type of adipositas genitalis either never menstruate or as soon as the disease develops, become amenorrhoic.

An interesting case of traumatic pituitary disease has been studied. J. J., 29 years of age, 12 years previously sustained a severe head injury. Menses ceased, polydypsia, obesity, eye symptoms developed. Although relieved of her pituitary tumor (cyst) by transnasal operation and radium, the polydypsia and polyuria (9 litres in one day) and amenorrhea have continued. Our blood studies have shown no cycle.

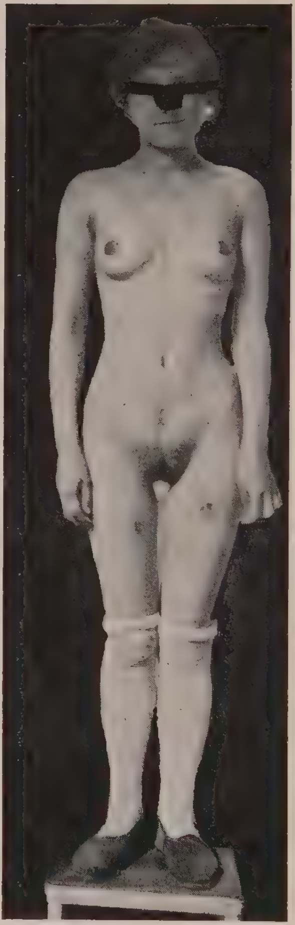


Fig. 80. Case of Absence of Vagina.

Showing feminine configuration and secondary sex characters. This patient has a 21 day blood cycle. (No. 1399).

Cases of atypical obesity with hirsutes and amenorrhea are difficult to differentiate from the foregoing. Some of these patients show a subthreshold blood cycle (see page 216). I consider hirsutes of bad prognostic significance.

A case of true acromegaly (See Fig. 81) first showed excessive menstruation and then became amenorrhoic.

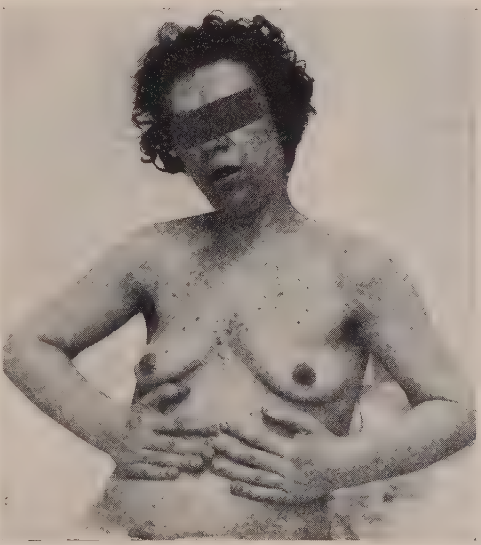


FIG. 81. ACROMEGALY AND VON RECKLINGHAUSEN DISEASE.

Typical acromegaly ushered in by menorrhagia, later replaced by metrorrhagia.

Thyroid Disease

Hyperthyroidism is almost invariably accompanied by menorrhagia in its early stages. Later amenorrhea develops. This holds true of both Graves disease and so-called toxic goiter. Frequently the gynecologist is called upon to make the diagnosis, as the disturbance of the menstruation attracts the patient's attention to the exclusion of other symptoms.

MENORRHAGIA: UNDIAGNOSED HYPERTHYROIDISM

Thirty-two years; E. S., bleeding two months, unenlarged thyroid; no heart or intestinal symptoms; lymphocytes 35 per cent; basal metabolism +61.7 per cent; one month later +56 per cent.

Deficient thyroid secretion is followed by amenorrhea.

AMENORRHEA: MYXEDEMA

Twenty-nine years, D. C., increasing oligomenorrhea, now amenorrhea for one year; has not gained weight; feels sluggish and sleepy. Skin coarse and dry. Basal metabolism −33 per cent. On six grains of thyroid substance began to menstruate after 3 months.

Adrenal Disease

No cases of Addison's disease have been observed by me.

The patient whose picture is shown (Fig. 62), in addition to advanced von Recklinghausen's fibroneuromatosis, shows the asthenia and debility characteristic of advanced adrenal hypofunction.

Pancreatic Disease

Diabetes may leave the menstrual function unaffected. Not infrequently, as shown by Rosenbloom (522) sugar tolerance is decreased during the menses. Even fertility is little affected by diabetes, these patients conceiving and bearing normal children.

Obscure Endocrine Conditions

In addition to the above readily grouped conditions, I have observed a considerable number of patients unquestionably suffering from endocrine disturbances but of such a type as to defy present classification. Combinations of acromegaloid stigmata, hirsutes, and obesity; a syndrome including eunuchoidism and hirsutes; obesity, pigmentation, and lymphocytosis, to instance only a few, proved most puzzling.

Virilism, Primary Amenorrhea: No Cycle

No. 1615, P. L., 26 years, married, 0 para, never menstruated; sex life normal. Mongoloid eyes, lips heavy, shoulders heavy, hips narrow; long arms and legs, big hands and feet; uterus small, pelvic outlet narrow; sellar X-ray normal, basal metabolism normal, sugar tolerance normal.

Blood studied, six specimens between March 12th, 1928, and April 22nd; no cycle. Prognosis poor.

Virilism: Primary Amenorrhea: Subthreshold Cycle

No. 1406, M. R., 26 years, single; never menstruated, male habitus, poorly developed breasts; large hands and feet, hirsutes. Normal vulva and vagina; small uterus, ovaries not felt. Sellar X-ray normal. Basal metabolism −6 per cent, sugar tolerance normal. Good blood cycle but because of the manifold stigmata a poor prognosis was given. This patient watched up to the present, has never menstruated. (X-ray to pituitary, spleen and ovaries, thyroid extract without effect).

For Graph 1406 see page 214

Obesity: Pigmentation: Lymphocytosis: Amenorrhea

No. 1188, F. R., 26 years; 2 children. Five years ago appendix removed and both ovaries resected for "cystic condition." This was followed by amenorrhea, tremendous gain in weight, now 225 pounds from 140 pounds. Pigmentation of arms and abdomen. Lymphocytes 60 per cent; basal metabolism normal. Menstruates occasionally.

For Graph 1188 see page 224

I have now observed a number of patients whose ovaries were resected for various indications in whom amenorrhea and obesity have subsequently developed. In a number of very young women on whom the same operation has been performed for puberty bleeding, no relief resulted.

Hence this procedure does not appeal to me, although good results have been reported.

HIRSUTES

From these few instances it is evident that amenorrhea, oligomenorrhea or even menorrhagia (usually of short duration and succeeded by hypofunction) may be secondary to the underlying endocrine disturbance. Therefore the gravity of the primary condition and its curability are the important factors in making a prognosis, and in deciding upon the treatment if such is feasible.

Hirsutes has resisted all of our efforts at endocrine therapy, not responding in the slightest to prolonged injections of female sex hormone extracts. We therefore at once advise the patient to undergo electrolytic treatment for the facial deformity resulting from this condition.

The condition is uninfluenced by thyroid therapy, or reduction in weight, if obesity is also present. The first sign of hirsutes often shows as a male skutcheon superadded upon the typical female pubic distribution of hair. The female hair ends in a curve with concavity upward, the male extends in the form of a triangle with its apex at the pubis (see Fig. 82). A moustache appears in some cases. Still rarer is a full beard as shown in Fig. 83. Intermediate in frequency is hair on the chest, thighs, and arms.

The pigmentation noted in certain cases of endocrine disease appears to have no connection with the genital sphere, although the patient's attention is attracted to the increasing discoloration at the time that amenorrhea becomes manifest. Therefore it is frequently regarded by them as due to the amenorrhea. No treatment has been of avail.

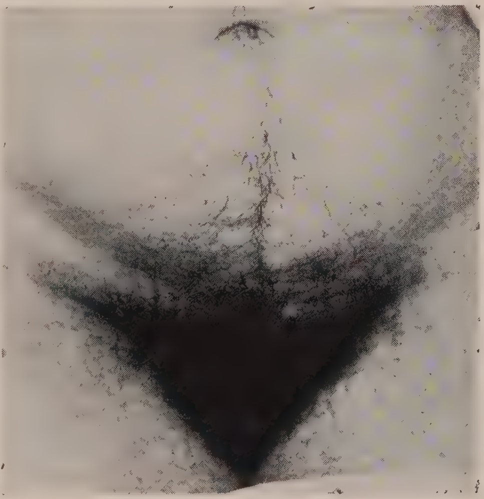


FIG. 82. MALE PUBIC HAIR DISTRIBUTION SUPRAPOSED UPON TYPICAL FEMALE PUBIC DISTRIBUTION.

This combination is seen not infrequently when hirsutes develops in adult life.

Facial Hirsutes: Virilism: Oligomenorrhea

No. 1262, Y. K., 26 years, married 5 years; 0 para; at age of 21 years menses became scant and appeared every 4 to 6 months; hair grew on face (grows 1.5 cm. in 7 days); blood examination over five weeks negative; basal metabolism —12 per cent; could not tolerate even small doses of thyroid extract; weight normal, 120 pounds; hair growth unaffected by long continued daily injections of 3 R.U. of female sex hormone. (Fig. 83).

Facial Hirsutes: Obesity: Acromegaloid

L. P., 33 years; 1 child, ten years ago; after pregnancy gained 88 pounds, now weighing 195 pounds. With this, irregular, scant menses and marked hirsutes, especially of face. Blood chemistry, blood count, and sugar tolerance normal. Basal metabolism −12 per cent. Virile fascies, pigmentation of vulva. Female sex hormone in blood negative. Thyroid therapy as well as long continued female sex hormone therapy proved ineffectual.

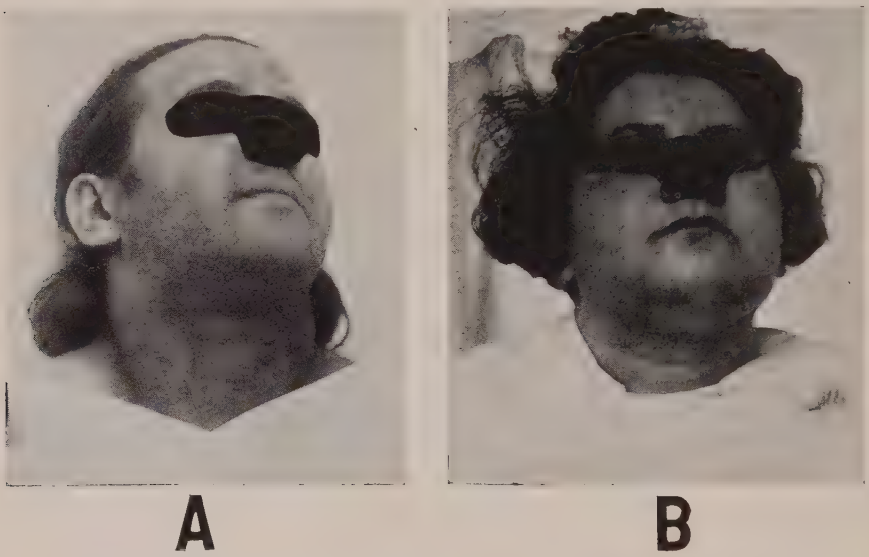


Fig. 83—A and B. Fully Developed Beards in Amenorrhoic Women.
A shows marked virility in features, strong neck and masculine demeanor.

Acromegaloid: Pseudohermaphroditic, Hirsutes

S. T., 29 years; operated 4 years ago for "fibro-cystic ovaries"; never menstruated. Breasts small, umbilical hernia; labia resemble split scrotum, miniature vagina, aplastic uterus, pelvis neuter. Sella turcica normal; basal metabolism normal ; sugar tolerance normal. Female sex hormone tests covering five weeks negative. Prognosis bad.

4. HERMAPHRODITISM

A bisexual tubular anlage is normally present in every mammalian embryo (see page 9). Pathological stimuli as well as experimental stimuli have succeeded in reversing the constitution of ovarian into testicular tissue (for details see Goldschmidt, 187). Occasionally individuals are encountered in whom either on one side is found a gonad of one sex, on the other the gonad of the opposite sex, or in whom the constituents of the gonads of both sexes are assembled in a single gland then known as an ovotestis. (See Fig. 84). Approximately 11 individuals are on record in whom the physiologically active constituents of both sexes were present, (see Lacassagne, 260; Kwartin and Hyams, 508), the ovarian portions

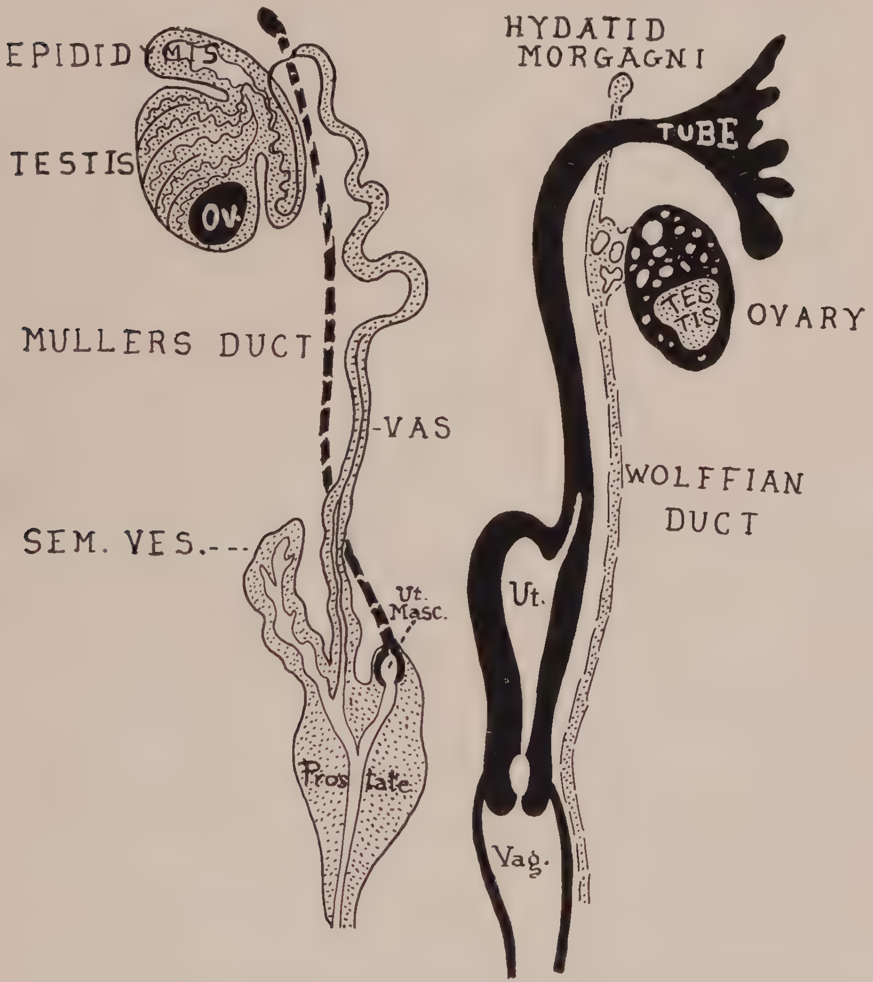


FIG. 84. SCHEMATIC OUTLINE OF THE GENITALS OF A TRUE HERMAPHRODITE.

On the left the male tubular system is in the ascendant, the Müllerian Duct being vestigial. On the right the Müllerian system is well developed, the Wolffian Duct being represented as the Hydatid of Morgani, ep- and par-oöphoron and the Wolffian Duct (Gärtner's Duct). On the left there is a testovar, on the right an ovotestis. Such individuals have been described, for example, the case of Lacassagne.

of the gonad containing ripening follicles and corpora lutea, the testicular portions containing spermatogonia. Under these conditions portions of both tubular tracts are usually developed to a considerable degree, the commonest combination being uterus and Fallopian tubes, prostate and penis which may imperforate.

More common are the pseudo-hermaphrodites in which the gonad of one sex alone is present, portions of the tubular tract of the opposite sex being hypertrophic (pseudo-hermaphroditismus masculinus or femininus internus and externus). In a few instances hyperfunction of the superrenal gland during intra-uterine life has been regarded as the cause. Usually the internal organs are female, the external organs male. A prostate is regularly present (Feldmann, 498).

I have had no opportunity of studying the blood of true hermaphrodites.

CHAPTER XIV

PHARMACOLOGY OF OVARIAN EXTRACTS AND FEMALE SEX HORMONE

The Basal Metabolism

IN THE earlier work performed with ovarian organotherapeutic preparations, much attention was devoted to the effect produced on the metabolism, and yet, although this feature was stressed in determining the efficacy of a given preparation, no unanimity exists about the fundamental relations between the basal metabolism and the gonads.

Basal Metabolism of the Male and Female Compared

According to Du Bois, page 347 (496) there is a lower basal metabolic rate (whether calculated by means of the surface area or the body weight) in the female than in the male. The author is inclined to ascribe this partly to a greater amount of body fat in the female, as well as to less muscular activity. That some other factor must also play a rôle is acknowledged by Du Bois from the fact that even in the adolescent age, when the girl exercises as much as her brother, her basal metabolism is somewhat lower. Stephani (525) says that the gaseous metabolism of the male compared to that of the female is as 6.16 c.c. is to 4.8 c.c.

Cyclical Influences

It is known that the difference between male and female metabolic rate becomes evident even before puberty. The reports on the effect of the cycle are so contradictory that little reliance can be placed upon the literature. Zuntz (528) found no change during menstruation. Lanz (511), on the other hand, found a premenstrual increase. Stephani (525) during menstruation, itself, found a decrease of 14 per cent which corresponds more or less to the figures of Lanz. A review of the literature will be found in Du Bois (496), page 349.

Effect of Castration

Loewy and Richter, in 1899 (319) noted a diminution in oxygen consumption of as much as 20.5 per cent in the castrated female dog. With oöphorin tablets an increase of 37.6 per cent above the normal (or 67.7 per cent above the castrate level) was determined, and yet, Lüthjé (513), in a carefully conducted experiment in 1903, could not verify the findings of the previous investigators nor could Zuntz, (528) in 4 women studied before and after castration, determine a decrease following the oöphorectomy or any effect produced by oöphorin. More recently, Murlin and Bailey, 1917 (345) determined a diminution of 14.2 to 17.5 per cent in the basal metabolism of 2 castrated female dogs. One of these dogs had a year previously been deprived of its thyroid so that any indirect effect exerted by the ovaries upon the thyroid gland could be excluded. These authors considered the diminution as due to extraneous factors, such as the sluggishness following oöphorectomy and the growing accustomed to the tests by the animals rather than to strictly ovarian influences. McCrudden, 1910 (332) in a most careful study of the metabolism of nitrogen, sulphur, phosphorus, calcium, and magnesium did not find any general retention of material, especially of the mineral elements of the body following castration, nor did he note any decrease in oxidation caused by the intervention. Quite recently Geist, Goldberger, Reiss, and Lande (177) have studied the basal metabolism, the weight, and blood chemistry preceding and following bilateral oöphorectomy in a series of 48 women. They found no consistent variation of the basal metabolism and body weight, nor did they find any change in the blood chemistry or blood pressure.

It would thus seem that the metabolic changes following castration are not very striking. However, in the main, a slight reduction in general metabolism has been noted by most observers following castration. On the other hand, the effect noted at the onset of puberty, during menstruation and at the onset of the menopause, have been either so slight or so variable as to defy analysis, by our present methods, and can therefore not be utilized in drawing warrantable conclusions. All in all, therefore, gauging the effect on the metabolism of an ovarian preparation, cannot be regarded as a reliable method of assay, even if Laqueur *et alia* (268) have conducted convincing experiments upon the castrated rat. (See page 109).

HISTORICAL

It is a matter of interest that as early as 1893 Regis (519) of Bordeaux made a Berkfeld extract of ovaries which he injected for the cure of insanity following upon operative menopause. Landau (510) in 1896 used desiccated ovaries for the treatment of castration and menopause symptoms. Poehl (see Aschner, 27) of St. Petersburg prepared both powdered and aqueous extract of desiccated ovaries. Since then such preparations have been marketed by innumerable firms. No uniformity of preparation has been observed. In some instances, the material has been defatted in order to make it more palatable, less disturbing to the digestion, and less easily decomposed. At present most pharmaceutical houses attempt to perform all processes *in vacuo* and at a low temperature.

PHARMACOLOGY OF DESICCATED OVARIAN PREPARATIONS

I have repeatedly put myself on record (1910, 1924, 1927) as entirely sceptical concerning the effect of desiccated, unconcentrated ovarian preparations. The so-called poisonous effect of corpus luteum and other ovarian preparations, when injected into the vascular channels was shown by me to be due to intravascular clotting (see page 57). The effects on the basal metabolism of oöphorin and similar desiccated ovarian powders have never given uniform results and have not stood the test of careful scrutiny and repetition. I have, during the course of a number of years, tested the marketed preparations biologically and found them, one and all, wanting in efficacy. The subjoined table gives a summary of these investigations. Geist and Harris (1923) (178) found the same thing. Loewe (and Lange) (314) was unable to obtain any biological reaction in corpus luteum preparations purchased or which he himself manufactured. Zondek and Bernhardt (490) too were unable to find any activity in the marketed preparations which they tested.

And yet the literature teems with glowing reports of the efficacy and the wonderful results obtained with these same biologically inert preparations. These effects vary from the immediate induction of menstruation after long periods of amenorrhea, the complete abolition of annoying and distressing menopause symptoms such as flushes, dizziness, and sweats, to the complete relief of intractable vomiting of pregnancy, etc., etc. I am unable to account for this unreconcilable discrepancy between titer and clinical results, except on the basis of psychical effect, lack of strict criteria and control, and the optimism of both patient and physician.

The following desiccated ovarian preparations were tested by both the growth of the rabbit's uterus method and the vaginal smear method. Huge doses were given subcutaneously. In case of tablets, these were finally powdered, emulsified with water and injected.

Pharmaceutical Preparations Tested

Armour's ovarian substance—neg.
Burroughs, Wellcome, "Varium"—neg.
*"Ciba"—Sistomensin and Agomensin—neg.
Hynson, Westcott's Lutein tablets—neg.
Iscovesco's Gynocrinol-Hypo—neg. Mouth tablets—neg.
Lederle's Corpus Luteum sol.—neg.
Lehn & Fink, Ovarian substance—neg.
Parke, Davis & Co.—ampoules corpus luteum—neg., ovarian substance-neg.
Reed & Carnrick Ovocoid Pills—neg.

The following anecdote will illustrate my own position in the matter. I recall driving to Brooklyn with a colleague of mine in 1927, a well-known gynecologist and obstetrician, who was accompanying me to listen to a talk on the female sex hormone which I was to deliver. We whiled away the tedium of the trip by discussing organotherapy. Dr. H. asked me whether I knew anything concerning a new water soluble potent female sex hormone just put on the market. I told him that I had just completed a rather careful assay of this product and found that although when quite fresh it produced a weak biological reaction, that after 2 or 3 weeks, all trace of efficacy had disappeared. "But" said I, "I am quite sure that the firm who has put this product on the market is already receiving glowing reports of the value of this preparation." As it chanced, the next day the director of the Research Laboratory producing the preparation in question, called me up and asked me whether I had completed my assay. When informed of my disappointing and completely negative findings, he expressed the utmost surprise, because he had just received most glowing reports of the wonderful effect of this preparation on 10 patients on whom it had been tried. The clinical work had been performed in one of the well known large hospitals of the middle west, by specialists. The Director then had the assay repeated in his own laboratory with the same negative result. How can one reconcile such unreconcilable contradictions?

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* Zondek (481) also was unable to demonstrate any activity. Uhlmann (526) on the other hand, working in the experimental laboratories of Ciba, describes positive results with Sistomensin.

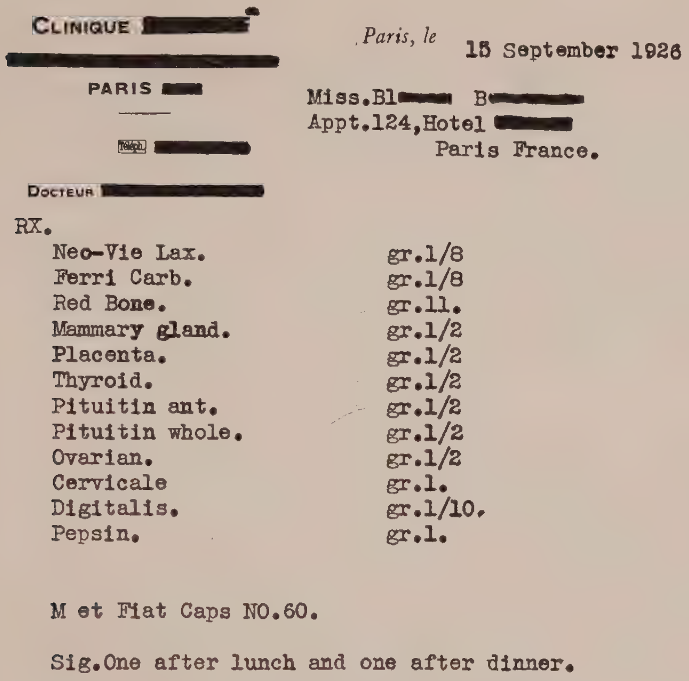
Biological Standardization

Quite recently the pharmacology of the female sex hormone has been much improved by the fact that the profession at large demands at least some semblance of titration and assay. Such varied methods as the effect on the basal metabolism (Laqueur, Hart, and de Jongh, 268), upon the muscular activity Slonaker, (417; Durrant, 103), upon the blood calcium, (Mirvish and Bosman, 516), and upon the blood sugar (Kylin, 509) have been studied and considered as a possible means of standardization. However, there is general agreement that the effect upon the growth of the uterus or the standardization by means of a rat or mouse unit must be insisted upon to judge of the efficacy of the preparations. Laqueur, Hart, and de Jongh (268) have performed very careful experiments in which they used six castrated rats for each experiment and in each test found that there was a distinct increase in the gaseous metabolism when active female sex hormone was injected in the test animals. The increase was from 2.70 to 2.80 grams CO_2 per Kg. animal hour to 3.10, under the influence of the extract. No effect on the male castrates was noted. Zondek, B. and H. (Med. Klinische 1926, 52:2016) described a similar increase in the human female castrate injected with water soluble female sex hormone. In parenthesis, I may add that Zondek, B. and Bernhardt, H. (490) the previous year had already obtained 12.5 per cent in the increase in the basal metabolism with an ovarian powder (ovornan)!

Kylin (509) found an increase in the blood sugar of animals injected with follicle extract, but a decrease of the blood sugar when animals were treated with corpus luteum extracts. From this he deduced that the corpus luteum extracts contained insulin-like substances.

Mirvish and Bosman (516) noted a uniform fall in the blood calcium, reaching 35 per cent as the result of the use of follicle fluid, corpus luteum, or placental extracts.

King and Patterson (507a, 507b) on the other hand, in a carefully controlled study of the basal metabolism in the menopause, never found more than 3.6 per cent of increase as a result of organotherapy.



CLINIQUE [redacted]

[redacted]

PARIS [redacted]

Téléph. [redacted]

DOCTEUR [redacted]

Paris, le 15 September 1926

Miss.Bl[redacted] B[redacted]
Appt.124,Hotel [redacted]
Paris France.

RX.

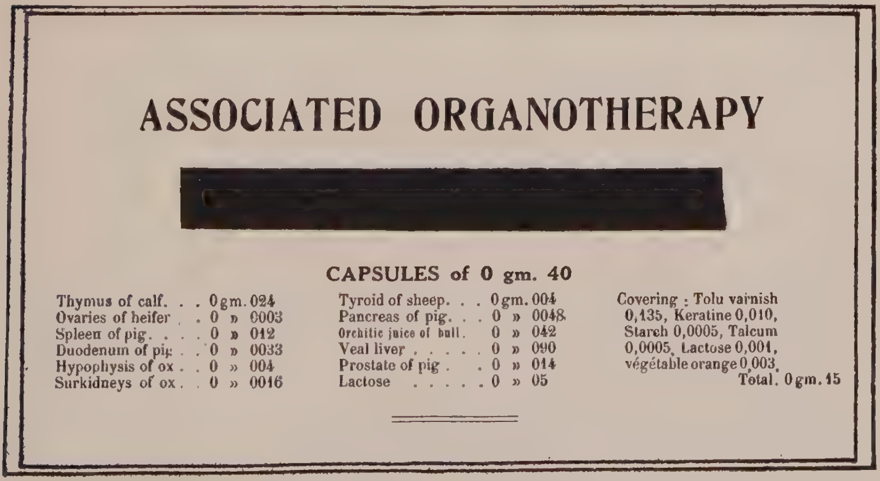
Neo-Vie Lax.	gr.1/8
Ferri Carb.	gr.1/8
Red Bone.	gr.11.
Mammary gland.	gr.1/2
Placenta.	gr.1/2
Thyroid.	gr.1/2
Pituitin ant.	gr.1/2
Pituitin whole.	gr.1/2
Ovarian.	gr.1/2
Cervicale	gr.1.
Digitalis.	gr.1/10.
Pepsin.	gr.1.

M et Fiat Caps NO.60.

Sig.One after lunch and one after dinner.

FIG. 85. SHOTGUN ORGANOTHERAPEUTIC PRESCRIPTION.

This prescription came into my hands through the kindness of the lady to whom it was dispensed. A ½ grain thyroid tablet would be equally efficaceous.



ASSOCIATED ORGANOTHERAPY

[redacted]

CAPSULES of 0 gm. 40

Thymus of calf. . .	0 gm. 024	Tyroid of sheep. . .	0 gm. 004
Ovaries of heifer . .	0 » 0003	Pancreas of pig. . .	0 » 0048
Spleen of pig. . . .	0 » 012	Orchitic juice of bull.	0 » 042
Duodenum of pig . .	0 » 0033	Veal liver	0 » 090
Hypophysis of ox . .	0 » 004	Prostate of pig . .	0 » 014
Surkidneys of ox . .	0 » 0016	Lactose	0 » 05

Covering : Tolu varnish 0,135, Keratine 0,010, Starch 0,0005, Talcum 0,0005, Lactose 0,001, végétable orange 0,003.

Total. 0 gm. 15

FIG. 86. PHARMACEUTICAL ENDOCRINOLOGY.

All of the above is contained in a capsule weighing only 0.4 (6 grains)!

Little is to be gained by either analyzing, abstracting, or, as I would be inclined, ignoring the huge casuistic literature dealing with the wonderful effects of ovotherapy. I have sufficiently often and strongly expressed my own views on the subject. It is really unfair to blame the pharmaceutical houses for featuring the literature, which testifies to the effects produced by preparations demonstrated as inert as far as content of female sex hormone is concerned. The fault lies with the medical profession and not with the manufacturer.

The extremes to which both the profession and the pharmaceutical houses go is best illustrated by Figs. 85 and 86, the first presented to me by a patient to whom this prescription had been given, the second received as third class mail.

Effects Produced by Potent Female Sex Hormone

The results obtained by Zondek and Aschheim (481) in twice inducing menstruation in a woman castrated some two years previously, by means of the injection of their specially prepared female sex hormone, appears well substantiated and credible to me. The report of Pratt and Allen (378) and of Allen (5) of having induced menstruation in castrated monkeys by means of the injection and the then abrupt withdrawal of female sex hormone extracts, likewise appears well authenticated. With these exceptions, none of the results so far published will bear critical scrutiny. The return of menstruation after prolonged amenorrhea, as I have tried to show, occurs frequently quite independent of any medication or treatment. The same applies as well to the relief of menopause symptoms, the vomiting of pregnancy and similar symptoms which cannot be fully evaluated nor critically controlled.

This criticism applies as well to some of my own therapeutic efforts, in which I employed female sex hormone prepared by my collaborator, Prof. Gustavson in Denver. This preparation was free of nitrogen, sulphur, and phosphorus, contained approximately 3 to 5 R.U. per c.c. of preparation in oil, the solid substance per R.U. approximated 2 and 3 mg. In cases of amenorrhea without cycle, injected daily over long periods of time, fullness and tingling of the breasts as well as pelvic fullness was periodically noted. Moreover the uterus would increase in size during the treatment but menstruation was not produced. Amenorrheas with cycle were not treated as here other factors would becloud the issue.

The most striking results were noted in 3 cases of kraurosis vulvae in all of which, after 10 days of daily injection, a marked amelioration of the subjective symptoms of dryness and itching were noted by the patient. Examination of the local findings also evidenced the return of moisture, succulence, and more normal appearance of the external genitals. Interruption of the injections was followed by a relapse which, in turn, could be controlled by renewal of the injection, but no permanent relief of symptoms could be obtained.

Even such striking effects are mentioned merely as a matter of record but are not accepted nor produced as conclusive indices of potency.

A study of the newer preparations offered to the profession in the last year or two, has been made by Frank and Goldberger (unpublished) see page 272. No claim at minutely accurate titration is made. However, our results are sufficiently conclusive to warrant our pointing out that the preparations now obtainable do not as yet meet the needs of practice, nor do they fulfil the requirements of the most lenient possible standard that we may set up. None of the preparations, to use a comparison, approaches what we require and demand in thyroid therapy or in the treatment of diabetes. Everything points to the fact that before much time has elapsed, the concentrated and continuous effort of many investigators will produce a usable and valuable female sex hormone preparation.

Effects of Different Methods of Exhibition

The mode of exhibition plays a big rôle in the effects produced. Subcutaneous injections appear to be the most effective method. If water soluble products are used, repetition of small amounts as, for instance 3 times daily, are more effective than a single subcutaneous dose. Oil media apparently allow of slower and more continuous absorption, but carry with them the drawback of occasionally producing persistent granulomata at the site of injection.

In test animals, intraperitoneal injections have been found much less effective than subcutaneous (Evans and Burr, 109).

By mouth the effect has been found to be one-twentieth to one one-hundredth less than by the subcutaneous route (Laqueur, 264; Loewe, Lange, and Faure, 1926, 315; Frank, 146).

Laqueur, Hart and de Jongh (269) have prepared suppositories containing as much as 500–1000 M. U.

These facts must be kept in mind in determining upon methods of treatment. For example, if a preparation contains 25 R.U., given subcutaneously, this might be expected to produce some effect, but by mouth an effect of less than 1 R.U. may be anticipated.

Either the female sex hormone after ingestion is absorbed with difficulty or, as I suspect, when it enters through the portal system, it is taken up in the liver and stored in the bile as mentioned on page 120. It therefore does not reach the general circulation and through this the genital system, unless huge doses are given.

Species Sensitivity to the the Female Sex Hormone

The utmost confusion reigns as to the dosage to be employed in the use of the female sex hormone. Comparison between mice and rats has shown that the ratio between mouth and subcutaneous dose in rats is as 1:100, in mice as 1:11 (Laqueur, 269) while the subcutaneous dosage in rats and mice is as 4:1, i.e., 4 M.U. = 1 R.U. although the weight of a rat is 8 to 10 times that of a mouse. What dosage is needed for the human being is still unknown.

The dose by the subcutaneous route will depend to a great extent upon the rapidity of excretion (urine, feces) which has never been determined. McClendon (515) reports having given from 2000–4000 M.U. to women, but, from a personal communication, I am unable to judge whether he took the labelled number of units at their face value or retitered the solutions used. At all events, these doses produced no disturbing symptoms. Loewe (Kl. W. 1927, 6:59) believes that 2000 M.U. must be injected daily to obtain an effect. Laqueur (269) says that 80–800 M.U. "may prove satisfactory." On the other hand, it has been shown that the human subject is much more sensitive to pituitrin and to insulin, for example, than some of the lower forms, and that a weight ratio may therefore not be applicable. More data must be accumulated before any attempt at generalization can be made.

PATENTS

A number of patents have been taken out, based on methods of obtaining the female sex hormone. None of them appears to have been exploited; not one of them contains anything of importance or value. For the sake of completeness I append reference to those that I have been able to find in the literature.

George M. Hieatzman, of Baltimore County, and William I. Hieatzman, of Baltimore, Maryland. Process of Separating Ovarian Product from its Surrounding Membrane. 1,163,538. Patented Dec. 7, 1915. U. S. P.

L. Seitz, H. Wintz, and Fingerhut. D. R. P. 332,165; 1916–25–I; also 332,165; 1916–25–I.

Ludwig Seitz and Herrmann Wintz, of Erlangen, Germany. Process for the Manufacture of a Menses-Increasing Substance from Corpus Luteum. 1,318,698, and 1,318,699. Patented Oct. 14, 1919. U. S. P.

Sigmund Fränkel and Edmund Herrmann, of Vienna, Austria, Hormones and Phosphatides and Process of Obtaining Same. 1,314,321, Patented Aug. 26, 1919. U. S. P.

Oliver Young Imray. Improved Manufacture of Physiologically Active Substances. 226,372. Dec. 24, 1924. Br. P.

A STUDY OF THE COMMERCIAL EXTRACTS OF THE FEMALE SEX HORMONE

Our study has been limited to extracts for which claims of biological titration have been made. In the United States, Parke, Davis and Co., and E. R. Squibb and Sons, to my knowledge, are manufacturing and marketing such products. If others are available, they have escaped my notice. Of the European products, my investigation has been limited to Menformon, old (4 M.U. to the c.c.) and Menformon, new (40 M.U. to the c.c.) prepared by Degewop, Gesellschaft Wissenschaftlicher Organpräparate A. G., and Progynon which in distinction to the others, appears in tablet form, and is marketed by the Schering-Kahlbaum A-G. The Squibb preparation was accepted by the Council on Pharmacy among New and Unofficial Remedies, under the title of "Ovarian Hormone Squibb." Recently Squibb, under the name of "Amniotin" have marketed a dark brownish preparation of unpleasant odor prepared from the amniotic fluid of cattle. This also was titred.

Hormovar brought out under the direction of Biedl, as well as Folliculin A and B for which Zondek and Aschheim are sponsors, I have been unable to obtain and test. Systomensin and Agomensin, prepared by the Ciba Co. have also been tested.

Without claiming to have performed the accurate assays now demanded by Laqueur, Coward and Burn as well as Kahnt and Doisy, as will appear from the appended data, my titrations have been sufficiently numerous to warrant definite deductions. As this subject is of such great importance to the practitioner, I do not hesitate to present some of the data in detail.

Estrogen, No. 157, May 15th, 1928.
Labeled 25 R.U. to the c.c.
Equivalent of 1 R.U., weight of rat 132 grams—result 0
“ “ 2 R.U., “ “ “ 140 “ — “ 0
“ “ 2 R.U., “ “ “ 132 “ — “ 2
“ “ 3 R.U., “ “ “ 140 “ — “ 2+
“ “ 5 R.U., “ “ “ 142 “ — “ 0

Estrogen, No. 2824142
Equivalent of 8 R.U. in a mouse—result 3
“ “ 4 R.U. “ “ “ — “ 0
“ “ 3 R.U. “ “ “ — “ 3
“ “ 12.5 R.U. “ “ rat, weight 232 grams—result 2+
“ “ 6.4 R.U. “ “ “ “ 178 “ — “ 4
“ “ 8 R.U. “ “ “ “ 208 “ — “ 3

Estrogen, No. 093563 A.
Equivalent of 6 R.U.—result 0
“ “ 8 R.U.— “ 0
“ “ 12.5 R.U.— “ 3
“ “ 12.5 R.U.— “ 3
Same sample 4 weeks later
Equivalent of 12.5 R.U.—result 0
“ “ 12.5 R.U.— “ 0
“ “ 25 R.U.— “ 0
“ “ 25 R.U.— “ 0

Estrogen, No. 2869692
Labeled 25 R.U. to the c.c.
0.5 c.c. equivalent of 12.5 R.U., weight of rat 190 grams—result 0
0.25 c.c. “ “ 6 R.U., “ “ “ 174 “ — “ 0
0.04 c.c. “ “ 1 R.U., “ “ “ 156 “ — “ 0

Squibb's Ovarian Hormone, No. 36844
Equivalent 3 R.U., weight of rat 170 grams—result 2
“ 5 R.U., “ “ “ 176 “ — “ 3+
“ 1 R.U., “ “ “ 150 “ — “ 0
“ 2 R.U., “ “ “ 122 “ — “ 2+

Test of No. 36844 2 days later.
Equivalent 1 R.U., weight of rat 150 grams—result 0
“ 1 R.U. “ “ “ 122 “ — “ 0
“ 3 R.U. “ “ “ 170 “ — “ 2
“ 5 R.U. “ “ “ 176 “ — “ 3+

Of the earlier preparations, No. 29721
3 R.U. to the c.c., 3 c.c., equivalent of 9 R.U.—result 0
No. 29615, 3 R.U. to the c.c.
1 c.c.—result 0
3 c.c.—result 0

No. 31670, 3 R.U. to the c.c. No quantity less than 3 c.c. gave any reaction.
Equivalent of 9 R.U.—result 4
No. 33526.
.4 c.c. gave 3
Amniotin Squibb
No. 39034, labeled 20 R.U. to the c.c.
.05 c.c., equivalent to 1 R.U., weight of rat 206 grams—0
.1 c.c., “ “ 2 R.U., “ “ “ 218 “ —0
.2 c.c., “ “ 4 R.U., “ “ “ 216 “ —0
.3 c.c., “ “ 6 R.U., “ “ “ 212 “ —0
.5 c.c., “ “ 10 R.U. “ “ “ 192 “ —1
1 c.c., “ “ 20 R.U. “ “ “ 180 “ —3

Systomensin, Ciba
Repeated attempts to produce any effects in rats failed. In 3 mice, each injected with 2 c.c., the reaction was negative. Needless to say, smaller quantities were likewise negative.

Menformon
No. 891, labeled 40 M.U. to the c.c.
Equivalent of 1 M.U.—result 0
“ “ 2 M.U.— “ 0
“ “ 2 M.U.— “ 0
“ “ 3 M.U.— “ 0
“ “ 5 M.U. “ 3

No. 1023, labeled 40 M.U. to the c.c.
Equivalent of 1 M.U.—result 0
“ “ 1.5 M.U.— “ 0
“ “ 1.5 M.U. “ 0
“ “ 2 M.U.— “ 0
“ “ 3 M.U.— “ 0
“ “ 5 M.U.— “ 0

No. 1721, labeled 40 M.U. to the c.c.

Equivalent of	1 M.U.—	result	0
" "	2 M.U.—	"	0
" "	3 M.U.—	"	0
" "	4 M.U.—	"	0
" "	5 M.U.—	"	0
" "	8 M.U.—	"	0
" "	12 M.U.—	"	0

3 c.c. equivalent to 120 M.U. in a rat—result 0

All previous readings in mice.

Progynon,

Labeled 250 M.U. per tablet.

1 rat was fed one-third tablet for 6 days, equivalent of 500 M.U., effect =0

1 rat was fed two-thirds tablet for 6 days, equivalent to 1000 M.U. =2+.

Progynon tablets were powdered and emulsified with water.

12.5 M.U. subcutaneously to a rat—result 3
20 M.U. subcutaneously to a rat—result 4

Smaller quantities did not produce any reaction.

Summary Table

MARKETED BY	TRADE NAME	R.U. OR M.U. PER C.C. AS LABELED	R.U. OR M.U. AS FOUND BY US
Squibb	Ovarian Hormone	3 R.U.	1
Squibb	Amniotin	25 R.U.	3–0
Parke, Davis	Estrogen	25 R.U.	2–0
Degewop	Menformon, old	4 M.U.	0.75 M.U.
Degewop	Menformon, new	40 M.U.	0
Ciba	Sistomensin	(2 c.c. in mouse) (6 c.c. in rat)	0 0
Schering-Kahlbaum	Progynon	250 M.U. pro tablet	by mouth 0 Hyp. 15 M.U. =1 R.U.

As will be seen from the above, these titrations are extremely disappointing, showing that even if the material left the hands of the manufacturer with the amount of mouse or rat units labeled, deterioration rapidly destroys most of the activity and makes the clinical use illusory.

Some of these preparations were sent directly from the laboratory of Squibb and Sons, and Parke, Davis and Co. and therefore were as fresh as could possibly be obtained. The Amniotin and Progynon were purchased in the open market. Ovarian Hormone Squibb, in large quantities, was supplied through the kindness of Squibb and Sons. I desire also to thank Dr. Lescohier of Parke, Davis and Co. for having supplied us with large amounts of Estrogen. Mr. Bardos of the Marvell Pharmacal Co. is to be thanked for having furnished us Menformon old and new for examination, and the Ciba Co. of New York for having supplied large quantities of Systomensin and Agomensin.

PRICE

At present the prices of the various ovarian hormones and female sex hormones are almost prohibitive. Ovarialhormon-Folliculin (Menformon, old) costs 10 marks 50 pf. for 1 ampoule containing 4 M.U. The new Folliculin of which each ampoule is said to contain 40 M.U., has been sold here at $5.00 for 3 ampoules. I paid $9.50 for 10 tablets of Progynon, each tablet presumably 250 M.U. Estrogen costs $4.00 for 6 ampoules, each ampoule labeled 25 M.U. Amniotin, Squibb, 5 c.c. (labeled 50 units) costs $3.00 and 5 c.c. (labeled 100 units) costs $6.00.

CLINICAL USE OF FEMALE SEX HORMONE PREPARATIONS

From what has preceded, it must be evident that the water soluble forms of the female sex hormone undergo rapid deterioration with loss of potency. In addition, our own experience has shown that severe local reactions develop at the site of injection. These reactions vary greatly in different patients. Both Squibb's Ovarian Hormone and Parke, Davis's Estrogen are borne without complaint by a number of patients to whom we have given it. Others absolutely refuse further treatment because of the severity of the burning, swelling and pain which result at the site of the hypodermic injection.

During the years 1927 and 1928 we (Frank, Goldberger, and Gustavson, unpublished) have given as many as 80 injections to the same patient, of female sex hormone dissolved in oil, without severe local reaction or unpleasant consequence. In oily menstrua the female sex hormone retains its potency for long periods of time. However, the knowledge that annoying and disabling granulomata may develop at the site of the oil injections has deterred us from further use of the female sex hormone dissolved in oily media.

Numerous clinical reports are beginning to appear in the literature concerning the efficacy of the female sex hormone. As previously mentioned, the report of Zondek (481) and Pratt and Allen (378) are the most convincing as the former obtained menstruation in one castrate human being and the latter in numerous monkeys. The clinical results obtained by Pratt and Allen (378), McClendon (515) and others, however, for the purpose of our discussion cannot be considered as more convincing than the excellent results reported with biological preparations, which we know contain no demonstrable quantity of female sex hormone, and must therefore for the present, at least, be disregarded. This applies particularly to the effects upon the symptoms of the artificial or spontaneous menopause, the production of menstruation in amenorrheas, the relief of sterility and other quite incalculable and uncontrollable effects. As I have attempted to show, amenorrhea is such a variable symptom complex that no treatment whatever, or non-specific treatment, may well be followed by the appearance of menstruation.

CHAPTER XV

TREATMENT OF FUNCTIONAL DISEASES OF THE GENITAL TRACT

THE evaluation of any treatment in purely functional conditions is extremely difficult and unreliable. In the first place such great uncertainty and variation in diagnosis is possible, that no comparison of the results obtained by different observers and clinicians is feasible. In the second place, chance plays such a tremendous rôle as again to defeat any attempt at statistical treatment. Therefore what follows must be regarded simply as a generalization and as an attempt to present to the reader my own methods and results.

In no chapter of medicine is the ancient adage of *nil nocere* more important than in this one. Untold harm has been done by reckless resection of the ovaries. In several instances in my experience so-called "stimulating" doses of X-ray to the ovaries have given the *coup de grâce* to ovaries whose function was already greatly impaired and subnormal. Repeatedly, uncalled for and unnecessary insufflation through an infected cervix has to my knowledge, by producing a pelvic inflammation, changed a potential sterility into a definite and incurable one.

PLANNING OF TREATMENT

In deciding upon what measures to take in a given case, it is of importance to catalog the patient in her proper group. The general constitution, the underlying basis of the disease, if this can be determined, and its duration must be taken into consideration. The gravity of the condition, particularly whether it is self-limited, transient, or progressive, must be determined.

HYPOFUNCTIONAL CONDITIONS

It is well to remember, as I have emphasized in the previous chapters, that we are at present not in the possession of a reliable organotherapeutic female sex hormone preparation and that our treatment therefore must be indirect. Of the endocrine preparations that can be relied upon in these cases, there is only one, namely thyroid. Occasions arise when posterior pituitary (pituitrin) may be of use as an oxytocic or to control polyuria. My own experience with insulin and parathyroid hormone in the treatment of uterine bleeding has been small and entirely disappointing. All anterior lobe pituitary preparations at present on the market or even those obtainable for experimental purposes, have proved inert in my hands. I therefore, as previously, object strongly to putting the patient to the expense and trouble of submitting to hypodermic or oral medication with these various inert endocrine preparations.

Because of this fact, I have found it of use to direct my attention to the treatment of the individual as a whole and also to pay particular attention to certain recognizable symptoms.

Age

The patients to be treated are adolescents, mature females, preclimacteric women or women already in the senium.

The average adolescent requires instruction, given through the parents, including relief from overwork at school, fresh air, well-selected food, alternating rest and exercise, protection from undue nervous strains, in weak, underweight or nervous girls. The fat, sluggish, Froehlich type of adolescents, on the contrary, require restriction of diet, occasionally thyroid medication, and are often benefited by being removed from a too indulgent family circle. The adolescent girl with enlarged thyroid may require appropriate iodine treatment.

Sufficient time has not yet elapsed to permit me to come to a definite conclusion whether appropriate prophylactic measures can be instituted in the adolescent with the result of overcoming the latent endocrine troubles which manifest themselves in more clear-cut fashion in the next decade. Doubtless as our armamentarium improves, particularly when potent endocrine preparations have been elaborated, we may hope to influence favorably the development of young women before they reach maturity.

In the mature female the stigmata which have only been nascent in the adolescent, become fully manifest and developed. We then have to deal with the normal feminine, the infantile, the eunuchoid or the masculine woman. Much will depend upon the amount of deviation from the normal. In the hypofunctional group the truly feminine woman is more subject to transient abnormalities than any of the other three groups. Amenorrhea due to too prolonged lactation, to marked increase in weight, or to seriously incapacitating illness, responds favorably to proper general therapeutic measures. On the other hand, in the infantile and eunuchoid group, general measures are rarely of avail, unless in the presence of obesity where they are directed to reduction in weight.

The preclimacteric individual falls either into the grouping of obesities, or into the so-called old maid type. In both, the hypofunctional climacteric troubles usually are amenorrhea and vasomotor symptoms of the approaching menopause. These manifestations resist practically all forms of treatment.

Weight plays a great rôle in determining the treatment of hypofunctional patients.

There is a large class of women, many of whom show stigmata of infantilism, who remain underweight for the first 4 decades of their existence. These individuals are thin-boned, soft-skinned, and have a low blood pressure, often between 100 and 110 systolic and down to 50 and 60 diastolic. If overfed and given rest cures, they will gain from 10 to 20 pounds under protest. Their appetites are poor and their digestion uncertain. As soon as they are again subjected to the ordinary stress of life, immediate loss of weight recurs, and the struggle has to be renewed. During the time of subnormality, such patients often do not menstruate. However, it should be remembered that a certain number of this type of woman also are found in the normally menstruating groups.

Overweight

This large and interesting group must be subdivided in several ways—young women with overweight and older women. Diffuse obesity must be distinguished from lower girdle obesity and the rare upper girdle obesity. The uncommon type of adipositas dolorosa has also to be taken into account. Only twice in my experience have I come across markedly overweight young women suffering from menorrhagia. The others all complained of amenorrhea of longer or shorter duration. A further subgrouping must be made in fat women whose basal metabolism is normal and those whose basal metabolism is below the normal. Unfortunately the former group predominates. It is much easier to reduce obese women whose basal metabolism is subnormal.

In the obesities much improvement can be obtained by judicious treatment. Too many physicians are content to give directions to the patient "to diet" and lose weight. It is necessary to enter into the minutiae of the dietary and to give most detailed directions in every case. It is my habit to limit the amount of weight to be lost to one pound a week as a maximum, otherwise I have found that patients will be weakened in the course of reduction and most of them lose courage and interest in the cure long before the desired result is obtained. Consequently I discuss their bills of fare with them in detail, try to reduce the quantity and withdraw fattening foods in particular. Furthermore, I regularly order that during one day in the week a more liberal diet be taken. Under these conditions the patient will cooperate over long periods of time.

In every instance of real obesity a cautious attempt to use thyroid is made. We start with small dosage, giving not more than one-half grain of Armour's thyroid substance (any other thyroid preparation with which the physician is familiar may be substituted for this) three times a day, with strict supervision of the pulse rate and caution to stop at the onset of symptoms of cardiac palpitation or pain. This beginning dosage is only exceeded in cases where a really substantial reduction of basal metabolism has been determined by a test. When more than minus 15 per cent has been found, 1 to 2 grains, 3 times a day are borne. In cases of minus 30 per cent, the patients will tolerate from 5 to 6 grains a day.

In a large number of cases no other treatment of long persistent amenorrhea has been needed than this combination of diet and thyroid therapy.

The Condition of the Blood

I have not seen a case of chlorosis in many years. Formerly this was a commonly encountered clinical entity with consequent hypofunctional genital symptoms. Simple or secondary anemia is much more common in the hypofunctional group.

Lymphocytosis is quite commonly seen in latent or overt hyperthyroid disease and therefore is noted in some of the secondary hypofunctional genital conditions consequent to this primary trouble. The average lymphocytosis is 30 to 35 per cent in these cases. I have noted as high as 60 per cent occasionally.

If the red blood count is persistently low and the hemoglobin extremely reduced, a transfusion may be resorted to. This is most often necessary in puberty bleedings.

Amenorrhea

This important symptom has been previously discussed and the amenorrheas were divided into those with no cycle, those with subthreshold cycle and those with such a high cyclical accumulation of hormone that impending menstruation could be predicted. Naturally the treatment will depend entirely upon the light thrown upon the condition by the female sex hormone test. This does not signify that we perform a test upon every case of transient amenorrhea which presents itself for examination. Frequently a sudden change of climate as immigration from Europe to the United States, a beginning tuberculosis, a well-marked endocrine disease, such as hyperthyroidism, are sufficient to account for the amenorrhea and make the test unnecessary.

On the other hand, we never resort to X-ray treatment of the ovaries in cases in which no subthreshold cycle has been found, as I have repeatedly felt that permanent and irremediable amenorrhea has been induced by injudicious raying, in patients whose ovarian function was at the time completely in abeyance.

In the few cases in which we have given female sex hormone preparations by the hypodermic route, no effects were obtained, even in those cases in which breast symptoms and pelvic heaviness resulted. The gratifying results reported by a number of clinicians who gave female sex hormone preparations have not as yet convinced me. In the near future, however, I expect that by means of potent preparations we may be able to load the endometrium to such a degree that sudden withdrawal will be followed by menstruation. Whether this will in any way help to re-establish the normal menstrual function appears doubtful to me, as all evidence favors the view that the ovaries themselves are unaffected by the female sex hormone. It is much more likely that when we obtain a potent anterior lobe pituitary preparation, real and valuable stimulation of the ovaries may be brought about, if the stage of hyperluteinization of the ovaries is avoided. Perhaps by this means the ovarian function may be permanently stimulated.

In general it may be said that amenorrhea is best influenced by general hygienic measures, reduction in weight of the obese, regular but not too frequent sexual intercourse, and in appropriate cases, which are particularly the so-called polycystic ovaries, X-ray stimulation.

Vasomotor Symptoms

Flushes, local and general, sweats, dizziness, urticarias, edemas, are noted not infrequently in hypofunctional conditions of all ages. The treatment is extremely unsatisfactory. Reassurance, careful hygiene of the skin, (alcohol rubs, boric acid powder), mild sedatives, avoidance of over-excitement, may give some relief. These symptoms, when noted after the operative, X-ray or physiological menopause, are even more resistent to treatment. It is hard to say whether the symptoms produced after hysterectomy should be classified as hypo- or hyperfunctional. The blood hormone level is ordinarily not high and the so-called symptoms of "tension" to be referred to again with the hyperfunctional group, seem absent. In my experience, sedatives, particularly bromides or luminal combined with small amounts of ergot (5 to 10 drops, 3 times a day) are of use. Attempts to combat these symptoms by means of female sex hormone have failed in my hands almost in every instance, although others report successes with every imaginable organotherapeutic preparation.

Sterility

It is of course impossible to discuss the manifold aspects of this con dition in the present connection.

I desire to protest most emphatically against the growing tendency to subject women to long, searching, and protracted examinations for sterility unless at least a sufficient time for impregnation has elapsed. Again and again I see young women who have been examined most minutely. On inquiry, however, I discover that even the husband's semen has never been investigated and, although the couple have been married for 3 or 4 years, contraceptive measures have been practiced until the preceding 3 or at most 6 months. Such a procedure is distinctly unscientific, to say the least.

After a sufficient time for impregnation has been allowed to elapse, and this is a variable time, depending on the age of the couple, (it should be longer with very young poeple, it should be shortened if the women is reaching the age of 33 or 35 years), the husband's fertility having been established, all recognizable inflammatory factors having been excluded especially the purulent cervical plug, and tubal stenosis having been ruled out, a careful evaluation of the patient must be made. The question of sex hygiene must also be inquired into. It is surprising how often too frequent coitus which rapidly weakens the quality of the semen, as particularly cattle breeders have shown us, avoidance of the optimum time of conception, and ignorance in some of the elementary technical details of coitus, are at fault. Let us take for granted that all of these extraneous factors have been ruled out. Then there remain at most some 30 per cent of patients in whom a true endocrine sterility may be demonstrated. These are the patients whose blood shows a low premenstrual hormone threshold as chart, Fig. 75 so clearly demonstrates. A prognosis may be arrived at based upon the number of anatomical stigmata, the co-existence or absence of marked endocrine disease, the level of the female sex hormone in the premenstrual blood, and the response to general hygiene, as I have indicated in a paper written in 1927 (Frank, 150).

The treatment may be readily divided into general hygienic and local. In case of obesity, restriction of diet and thyroid substance is at once indicated. In the underweight and subnormal group with low blood pressure and asthenia, increased rest and feeding as well as control of the sex life must be undertaken. I have a wholesome fear of stimulation of the ovaries by means of the X-ray. It is in this group of cases that I hope most from an anterior pituitary hormone in the future.

Such measures as the intra-uterine stem pessary, Dudley or Pozzi trachelorrhaphy for straightening the cervical angle, appear to me to be based on entirely wrong theoretical considerations.

Hypofunctional Bleeding

In the fewest instances I have encountered persistent bleeding in patients who unmistakably fall in the hypofunctional class. One of these patients was described in detail on page 233. Her bleeding had persisted for 5 years. Repeated blood examinations showed that no cycle (observed over 6 weeks) was present. This patient gave the impression of some not classifiable endocrine disturbance, a combination of acromegaloid status, hirsutes, and obesity. Therapeutic efforts were directed to reducing the weight, which responded very poorly to a combination of strict diet and thyroid. Convinced of the hypofunctional condition, a very mild series of X-ray "stimulation" to the ovaries was practiced, in spite of the absence of blood cycle, the uterine bleeding warranting this departure from our rule. From this time on, menstruation has become regular, after 5 years of bleeding. One year ago she conceived, but unfortunately aborted of twins at the end of the fifth month. She will attempt to conceive again.

Another instance is a girl weighing 240 pounds, at the age of 17 years, in whom persistent bleeding had been noted for 2 years. Reduction of the obesity, which required the strictest of diet and large doses of thyroid, was followed by normal menstruation.

Kraurosis Vulvae

Kraurosis vulvae is a hypofunctional disease. It is frequently, but by by no means always, associated with the menopause. This condition responds even to the weak female sex hormone preparations at present at our disposal, although unfortunately the improvement is only of short duration. Rapid local improvement is noted but it recedes immediately after the hypodermic injections are discontinued. As low a dosage as from 5 to 10 R.U. a day are effective. However, after withdrawal of the stimulus produced by the female sex hormone, I have been obliged in two cases to perform vulvectomy to relieve the intolerable vulvar itching.

ENDOCRINE DISEASES

As repeatedly mentioned, functional disturbances of the female genital sphere arise in the course of most endocrine diseases. Hypofunctional changes develop in the genital tract at the onset of myxedema, Addison's disease and Froehlich's pituitary syndrome.

Hyperfunction more commonly results at the beginning of hyperthyroidism, whether of the Graves, or toxic variety, acromegaly, occasionally also at the beginning of a diabetes. It is of importance to remember that the resulting menorrhagias are usually succeeded by amenorrhea, as in the later stages of all of these diseases, hypofunction usually supervenes.

The symptoms ascribable to the sex sphere, although striking, are usually transient, and therefore rarely require any treatment except such as may be directed toward the underlying endocrine trouble. Occasionally menorrhagia and metrorrhagia become so profuse that they may necessitate a local emergency intervention such as curettage.

HYPERFUNCTION

Hyperfunction may show itself by such general symptoms as "tension," or manifest itself locally by enlargement of the uterus and increased vascularity of the entire genital tract with functional bleeding.

Precocious Puberty

The rare cases due to ovarian tumors are permanently relieved by oöphorectomy. Removal of the offending growth is followed by regression of the secondary sex characters and return to the normal childhood condition until advancing years usher in physiological puberty. The rare cases of premature puberty due to adrenal or pineal disease regularly end fatally because of the underlying trouble. The still rarer cases of precocious puberty in which no demonstrable cause can be found, resist all known therapy, because, I believe, that no one has yet ventured to give X-ray treatment to the ovaries in such infants. Such therapy would be strongly contra-indicated because of the effects it might exert upon growth and development. The primary factor in these cases may be the anterior pituitary. I have been unable to follow such children through to later life and therefore I am not competent to prognosticate their future fate.

Puberty Bleeding

The comparatively small group that I have had the opportunity of studying shows few stigmata. No marked infantilism, no eunuchoidism, no great precocity have been noted. Only one of these patients was somewhat of the "fat girl" type. The general symptoms were due to the prolonged bleeding and consisted in anemia, weakness, and lassitude. In no case was a marked degree of under-nutrition observed. It is my habit to hospitalize these patients, to put them to bed under the best of hygienic conditions, direct sunlight, iron therapy, complete rest, good food. Even under these conditions the bleeding may persist. If marked anemia results, I resort to transfusion which has an excellent if only transient effect, but this effect may extend over long months in spite of continuous bleeding. In these cases insulin, parathyroid hormone, as recommended by Allen, Compere, and Austin (491a), styptol (cotarine phthallate), ergot, and similar drugs appear of no avail. We have been forced to resort to repeated curettages. The curettings show simple cystic endometrial hyperplasia and the intervention usually relieves for several months. Final resort must, in some cases, be had to X-ray. This should be cautiously given, first attempting to "tone down" the function. If this proves impossible, a temporary castrating dose must be given as a last resort.

At present I have one patient, 22 years of age, under observation, whose history of continuous bleeding began at the age of 14 years. At that time, after all other measures had proved of no avail, radium was inserted into the uterus by a prominent gynecologist. This gave relief for only 3 months. Since that time the patient has had almost continuous bleeding, year in and year out, relieved only by two periods of amenorrhea of 5 and 7 months duration while in Europe. This patient's general nutrition is good. Although of extremely nervous type and of nervous heredity, there are no stigmata. Her sex life is normal. She bleeds moderately throughout the month with probably one additional spurt of two days duration. Some four months ago I found her extremely anemic (hemoglobin 38 per cent) with a marked reduction of red blood cells and only 2000 white blood cells. The uterus was found normal in size and bleeding had not been relieved by curettages. The patient was therefore transfused and has since then been in excellent condition with a return to an approximately normal blood picture. She has just left for Europe in order to see whether this change of climate will again produce cessation of bleeding.

Maturity Menorrhagia and Metrorrhagia

Before a patient is placed in this group it is necessary to exclude uterine and cervical polypi, fibromyomata of the uterus, carcinomata, or other neoplasms of the genital tract, threatened or incomplete abortion, inflammatory lesions of the uterus and especially of the tubes and ovaries, including tuberculosis of the genital tract. In spite of the large groups which are excluded by the various conditions just enumerated, a large number of patients remain who, in general, impress as very normal women and include especially many multiparae.

The uteri of these patients vary within normal limits, but on the whole, are inclined to enlargement, fibrosis and turgidity.

Curettage which so often has to be practiced, as a precautionary measure, to exclude malignancy and sometimes in the hope of relieving the disability, usually shows normal endometrium, sometimes resting, sometimes indicating cyclical changes, and quite rarely showing stationary hyperplasia. Our efforts, directed to the treatment of the general condition, will depend upon the social status and activity of the patients. Rest supervision of high blood pressure and of cardiac troubles must of course be resorted to. Occasionally polycythemia gives rise to these bleedings.

Sex hygiene must be inquired into and supervised. In a certain number of cases coitus interruptus, sometimes recognizable by the resulting spasm and tenderness of the sacro-uterine ligaments, must be interdicted and other aberrations of the sex life corrected. The bowels should be kept open. A saline laxative, at least once a week, may be indicated.

Prolonged hot douches in the prone posture, although inclined to increase the trouble at the beginning of the treatment, if practiced conscientiously twice a day except during the menstrual period, frequently benefit the turgescence and improve or relieve the bleeding.

Drugs such as styptol, stypticin, ergot, pituitrin, insulin may be tried but are rarely of real value.

Curettage, as previously mentioned, may prove palliative, but is really undertaken mainly for diagnostic purposes.

In former days hysterectomy had to be practiced with surprising frequency. Because of the danger involved in hysterectomy, a number of years ago atmocausis (steaming out of the uterus with subsequent obliteration of the endometrium) was devised. I recollect on two occasions to have been obliged to perform vaginal hysterectomies on young nulliparous women below the age of 25 years. The introduction of radiotherapy has been of the utmost value and benefit in this connection. Some clinicians prefer radium for the reason that it can be introduced at the time of curettage and can be inserted by themselves. I have abandoned this method to a great extent, preferring X-ray. Occasionally the radium capsule has been known to slip from within the uterus and produce serious burns. In practically every instance an annoying leucorrhea persists for a considerable period after the use of radium. These drawbacks are entirely avoided by the X-ray.

X-ray, however, requires the proper high voltage equipment, an X-ray therapeutist technically fully trained, and is therefore limited to certain localities and larger cities. I have resorted to this method now for many years. In most instances it is wisest to prescribe sufficient dosage to produce temporary amenorrhea. It is difficult in cases of menorrhagia and metrorrhagia to "tone down" the ovarian action but this effort should be made in cases of nulliparae who desire children. To multiparae of 33 to 35 years of age, it is wisest to give a permanent, castration dose. In younger women, it will be found that even a strong dose is followed after 1 and one-half to 2 years by resumption of the menstrual function, which may then be perfectly normal in both periodicity and amount. To a small number of such patients, normal children have been born after resumption of the menstrual function.

Preclimacteric Hemorrhages

These patients usually require diagnostic curettage as submucous myomata and the still more serious condition of corpus carcinoma must be excluded. If only temporary improvement is obtained, early resort to X-ray castration may be had. Caution should be used only in those cases whose very labile nervous and vascular system warns us that castration will be followed by unusually stormy menopause symptoms.

Premenstrual Tension

An extremely interesting and puzzling group of cases is encountered in which the premenstrual tension is exaggerated. The usual tingling and fullness in the breasts rise to the point of agony. Debilitating sweats, explosive nervous phenomena, and irritability are complained of. In a few cases, study of the hormone level has convinced me that an unduly high threshold exists. Venisection of from 40–80 c.c. gives prompt relief, but is followed by immediate re-accumulation in the blood and therefore only postpones the evil hour which culminates at menstruation, and then is usually followed by prompt relief lasting for from 1 to 2 weeks. The customary drugs employed, such as sedatives, particularly bromides, afford some relief in mild cases. As these patients are almost always approaching the menopause, I no longer hesitate in giving either mild or castration doses of X-ray.

The cases in which subcutaneous hemorrhages, transient, localized or general edemas and other neurovascular symptoms assume disabling proportions have so far defied my therapeutic efforts. These patients are usually young women. If the X-ray is applied to the ovaries, unduly stormy menopause symptoms may be anticipated.

Fibrosis uteri, Fibromyomata uteri

I no longer hesitate to enroll these two conditions among the hyperfunctional ovarian states. The bleeding of fibrosis uteri can be relieved by castration doses of X-ray followed by only very slight reduction in size of the symmetrical hypertrophied uterus. Occasionally curettage gives temporary or permanent relief if X-ray is objected to.

The treatment of fibromyomata of the uterus does not fall into the frame of this monograph. In passing, curettage, myomectomy, hysterectomy, or X-ray castration may be referred to.

SUMMARY

In the therapy of functional diseases I rely on a preliminary careful general survey of the situation including the constitution of the patient, the age group in which she belongs, the particular symptoms to be dealt with, the functional group hypo-, hyperfunctional, and the special local conditions requiring attention. Of the organotherapeutic preparations, the sole one on which I really rely is thyroid substance. The other organotherapeutic preparations, occasionally employed, are really used for their drug-like and not their endocrine action. The main reliance is placed upon hygienic measures and in the hyperfunctional group, on the curet and the X-ray.

Fortunately nature very frequently seconds our puny efforts, if we do no harm and place the patient under the best conditions. Then those, in whom the poor endocrine inheritance or the acquired stigmata are not of too serious a nature, frequently respond to a gratifying degree. I never tell a patient, unless she has no uterus or no ovaries, that she can never have a child. In my earlier years I occasionally ventured such a prediction, and, I am glad to admit that time has proved me wrong in numerous instances.

CHAPTER XVI

SUMMARY AND REVIEW

PART I

IN ATTEMPTING to present as unfinished a subject as the female sex hormone, it proved necessary to take up in rather disjointed fashion various aspects of the entire question. In doing so, continuity and perspective were necessarily lost sight of in many instances. In order to counteract this fault, this short and summary review is offered at the close of the monograph, in the hope that a broad, continuous, and general picture of the question may be presented to the reader.

Future investigations will probably demonstrate that throughout the plant and animal kingdom, two, perhaps antagonistic growth substances occur, the male and the female sex hormone. The male hormone has only been investigated slightly because, as yet, no readily applicable and specific test has been worked out. The female sex hormone, on the other hand, is now more readily demonstrated, assayed, and concentrated.

Demonstrable in yeast and more concentrated in the sex organs of higher plants, the female sex principle can be isolated. In the animal kingdom the main site of the production of the female sex hormone is in the ovary of the female and in mammals, in the placenta. This female sex principle has, however, also been demonstrated in the blood, urine, and bile of males. To this anomalous finding further reference will be made.

At least four specific tests have been described for the recognition and assay of the female sex hormone. The most useful of these are the growth phenomena produced in the tubular tract of immature or castrate animals and the change in the vaginal spread of certain castrate rodents in response to the stimulation exerted by the specific substance. By means of these tests even small quantities of the active principle may be recognized.

The female sex hormone exerts its influence on the fetus in utero throughout pregnancy. In mammals a high level of female sex hormone concentration exists in the circulating blood. The substance has been demonstrated in both the amniotic fluid and in the cord blood of the fetus. Moreover, we have found that the liver through which the cord blood first enters, contains a far greater amount of the female sex hormone than other organs of the fetus. As our researches have never enabled us to demonstrate female sex hormone in the fetal ovary, the response and stimulation of the fetal genital tract must be ascribed to the hormonal effect derived from the mother. This stimulation shows itself in the growth of the uterus, vagina, and breasts in the female, of the utriculus masculinus, breasts, and prostate in the male. Immediately after birth the abrupt withdrawal of the female sex hormone produces activation of the newborn female tubular tract and of the breasts in both male and female neonatus.

Throughout puberty the ovaries exert a continuous but low level stimulus upon the female genital tract. This effect is demonstrated rather by inference than any positive evidence we have been able to adduce. Castration before puberty prevents the onset of this change and shows itself in marked involution or absence of growth in the tubular system.

Recently the onset of puberty has been demonstrated as an anterior pituitary effect which can be artificially produced by injection of anterior pituitary extract or the repeated implanation of fresh pituitary substance. This has, however, merely changed the point of inquiry, because no explanation is offered as to why the pituitary at this given time suddenly increases its activity.

As a result of this pituitary activation, a sudden increase in amount and speed of follicle ripening results. Complete maturation of at least one follicle takes place with rupture and consequent ovulation. Thereupon the follicle cavity undergoes the change to corpus luteum, and this yellow body flourishes. If no impregnation has taken place, after a short period, the corpus luteum involutes and regresses, persisting in the form of a corpus albicans, the residular scar in the ovarian stroma. On the other hand, if impregnation has taken place, the corpus luteum persists throughout the duration of pregnancy and does not involute until after the onset of labor. Thus two different cycles occur, the one, the short abortive or infertile, the other, the long fertile or pregnancy cycle.

In addition, we must keep in view a less dramatic but equally important process which is constantly going on in the ovary of all species, namely that of follicular atresia. In the human being, for example, only 1 in approximately 300 ova fully mature, rupture and offer a monthly chance of impregnation during the 30 years of sexual maturity. On the other hand, thousands of ova reach a moderate degree of maturation, produce a certain amount of follicle fluid and likewise undergo a certain degree of luteinization, which corresponds to a miniature corpus luteum. All evidence favors the fact that this process of atresia produces female sex hormone and plays a large rôle in keeping up the trophic nutrition of the genital tract.

The cyclical activity just described in the ovary, which shows itself in the infertile and fertile cycle, as well as the continuous growth occurring in follicle atresia, exert the dominant rôle on the tubular tract and breasts of the female. Therefore we must look for changes in the uterus, vagina, and breasts due to this ovarian activity.

In the infertile cycle, the uterus of the primate undergoes a regular repeated change, the most characteristic type being that of full menstruation. Here the inactive mucous membrane has a short period of rest, soon followed by an increase in the size, convolution, secretory activity of the uterine glands, which is increasingly marked as the mucosa passes through the interval and approaches the pregravid or premenstrual stage. If this pregravid change is balked of its object, which is the harboring of the impregnated ovum, an abrupt regressive change takes place which consists in rapid necrosis of the so-called functional layer (compacta and spongeosa). The ragged, raw, basilar layer then alone lines the uterine cavity and through this open wound the menstrual blood is poured out into the uterine cavity. However, the catabolic destruction may be much less marked, such blood as escapes sickering by diapedesis between the glands and through small breaks in the surface epithelium lining the uterine cavity. This process then resembles more closely the gradual involution of the lining membrane of the lower species such as, for example, that of the bitch and the sow in whom no menstruation occurs. Occasionally, at least in monkeys, menstruation has been observed to take place through an apparently resting mucosa.

In the fertile cycle the stages as described to and including the pregravid phase are identical with that of the abortive cycle. Because of the persistence of the corpus luteum (and not necessarily because of the presence and secretion of the unimplanted fertilized ovum) the mucous membrane, instead of undergoing the menstrual involution, continues its growth process and forms the decidua of pregnancy, in which the ovum is imbedded and which continues to line the uterine cavity until the onset of labor.

The mammary glands undergo a cycle comparable to that of the uterus, regression and involution occurring at an early stage in the infertile and progressing farther and to a more complete degree in the fertile cycle. In its most well-defined form the involutionary stage in the infertile cycle is marked by a secretion of colostrum, in the fertile cycle by the secretion of milk, if sufficient hyperplasia of the glandular tissue has taken place.

Experimental proof has been offered to show that the effect, both on the uterus and the breasts is directly ascribable to the female sex hormone circulating in the blood, which reaches the Müllerian system and the breasts through the circulation. The effect, up to a certain degree, appears proportional to the activating substance carried by the blood. The blood concentration, during the resting stage, which corresponds in the ovary to the beginning growth of the follicle, is extremely low. During the interval, the follicle is growing, secreting more female sex hormone, which is therefore found in greater concentration in the blood. The highest concentration, in the infertile cycle, is noted toward the end of the pregravid (premenstrual) stage at which time as much as 1 M.U. may be found in every 40 c.c. of human blood of the normal female. This time corresponds to the most flourishing condition of the corpus luteum. Abrupt withdrawal from the circulation of the female sex hormone is followed by an equally abrupt catabolic, necrotic, and involutionary change in the activated and hypertrophic uterine mucosa, which has been described as the desquamation of the functional layers. Where the menstrual process is not accompanied by this desquamatory process, but is more gradual, it seems likely that the "loading" of the endometrium has been less complete, or in rare instances, has not taken place at all, and that therefore the involution is not accompanied by loss of substance. I am unable for the moment to explain the mechanism of the transudation of blood under these circumstances.

On the other hand, if impregnation takes place, the corpus luteum for as yet unexplained reasons, persists. Menstruation does not occur and the uterine mucosa continues its hypertrophy to the degree observed in pregnancy. During the first 8 weeks of pregnancy the hormone blood level of the circulation is low. I am inclined to believe that at this stage the full amount of female sex hormone secreted by the corpus luteum is at once utilized and stored by the growing mucosa. Not until the rapidly growing chorion epithelium takes up the additional task of hormone secretion in sufficient quantity, is a high hormone level again detected in the blood. This regularly occurs after the 7th or 8th week of pregnancy and persists increasingly until the time of labor. A stupendous overproduction of hormone takes place. As much as from 5 to 10,000 M.U. may be excreted daily through the urine, not to speak of the as yet unstudied amounts excreted through the feces.

The mechanism which maintains and regulates the hormone level in the circulation is as yet hardly understood. During the menstrual cycle the variation may be described as being between 1 M.U. to 70 or 80 c.c. of blood in the resting period and rising as high as 1 M.U. to 30 or 40 c.c. of blood in the pregravid period of normal individuals. During this time not more than 1 to 10 M.U. per liter are excreted in the urine. In pregnancy after the first 7 to 8 weeks, during which the hormone level in the circulation as well as in the urine, appears to be low, a steady maintenance of 1 to 2 M.U. per 30 to 40 c.c. of circulating blood is noted until the end of pregnancy. The tremendous overproduction and consequent wastage which manifests itself by the excretion of from 5 to 10,000 M.U. per liter in the urine is unexplained. Such prodigality is greater than even the ordinary safety factor in nature would explain. The excretion in the feces has been only imperfectly studied. It may reach the astounding amounts of 30,000 M.U. per kilo of dried feces.

The female sex hormone or substances giving every test attributable to the female sex hormone, have been found in the blood, urine, and bile of males. This is a strange and apparently anomalous discovery, particularly as the female sex hormone exerts an antagonistic antimasculine effect upon the sex organs of the male. This effect is very striking in the immature individual, and, although less marked in the adult, is present. However, the female sex hormone noted in the circulation or urine of the male is neither constant nor of cyclical occurrence. Its occurence in the bile of senile males has led me to investigate the subject from a different angle. As has been shown, female sex hormone occurs in considerable quantities in almost all of the vegetable foods. In our average daily meals a quantity corresponding to many M.U. is ingested. However, when given by mouth, female sex hormone produces little if any effect upon the female tubular tract. I believe that the high female sex hormone content of the bile is due to a protective mechanism by which such female sex hormone as is absorbed through the portal system, is filtered out in the liver and reaches the bile where it is stored. If the concentration in the bile becomes too great, the surplus is excreted through the intestines and urine and may likewise transiently appear in the blood. Considerable experimental proof has already accumulated to bear out my contention.

So far I have focused my entire attention to the description of the female sex hormone. Increasing evidence has accumulated, at a rapid rate in the last few months, that the contention of the earlier investigators that a special secretion or special secretions are elaborated by the corpus luteum, holds true. The corpus luteum forms the central link, in what I have called the gestational gland, namely a compound and progressive gland of internal secretion, consisting of the growing follicle, the corpus luteum, and the placenta, all three of which, during their period of activity, elaborate and pour into the circulation female sex hormone.

Quite distinct from this secretion and analagous to the compound and multiple nature of other glands of internal secretion, such as the anterior lobe of the pituitary, the corpus luteum elaborates another secretory product. This, probably mainly water soluble incretion, has the function of sensitizing the mucous membrane of the uterus during the pregravid stage in order that it may respond to the irritation and invasion of the fertilized ovum by the formation of the maternal placental elements. Without this sensitization, the ovum cannot successfully take root and flourish.

In addition to this influence, the aqueous corpus luteum secretion likewise inhibits the growth of follicles and thus, under normal conditions, helps to produce the periodicity of the cycle.

We may therefore accept the presence of two sex hormones, the one, mainly fat soluble, the female sex hormone produced by the ovary, corpus luteum and the placenta; the other mainly water soluble, elaborated by the corpus luteum alone.

Evidence has been adduced which favors the view that every descendent of the germinal epithelium is able, if called upon, to produce female sex hormone. This may have its importance as an emergency substituting mechanism called out, for example, after the ovary has been subjected to castrating amounts of X-ray. During the period of recuperation the down-growing germinal epithelium appears already to secrete female sex hormone. By means of an almost microphysiological test it has been shown that not only the follicle fluid, but also the theca interna and the corpus luteum cells secrete, or at least contain, the active principle.

The chemical researches on the female sex hormone have been disappointing. The active principle, in its most concentrated form, appears to exert its influence in dosage even smaller than that ascribed to purified internal secretory products such as crystalline insulin, pituitrin, and epinephrine. As little as .001 mgm. of dry substance may contain a M.U. The hormone contains carbon, hydrogen, and probably oxygen.

It is extremely resistent to high degrees of heat, to strong acids and alkalies, to reducing agents, to catalysis, etc. On the other hand, it is extremely sensitive to oxygen and to light. The hormone is readily soluble in all lipoid solvents. It has been "dissolved" in water, but in such minute concentration that the question arises whether this is a true solution, even though the substance can be dyalized through collodion and animal membranes. When in a water phase it can always be recovered by treating with lipoid solvents such as ether. Moreover the hormone dissolved in water proves unstable in contrast to its stability in inert lipoid media, in olive and Mazola oil. It is cholesterol free and free of cholesterol reaction; double or triple bond absent; saponification resisted; OH group absent; C:O group absent; acid groups absent; basic groups absent.

We are justified in ascribing the phenomena of sex and reproduction to two hormones, the general female sex hormone elaborated by the gestational gland, and the more specific and less widely distributed, but for that reason not less important, corpus luteum hormone whose activity is limited to imbedding of the ovum and regulation of follicle growth. The action of the anterior lobe of the pituitary is so essential to the functional activity of the ovaries that it too must be considered as the primary sexual activator.

PART II

The bloods of more than 500 women have been studied by means of a simple test. The test consists of injecting the ethereal residue obtained from 40 c.c. of vein blood into a test mouse and reading the resultant reaction by means of the vaginal spread, on a scale from 0 to 4, in which 0 signifies no reaction and anything above 2+ a positive reaction. In addition, the menstrual blood and the urines of a number of patients were studied. This test, although not strictly quantitative, has given strikingly concordant results. The accuracy of the results depends upon the exactitude of the technic. In the normal woman a low hormone blood level exists until about the 7th day before the onset of the next menses. The rise produces a 3 or 4 in the test mouse. In a small number, the reaction, is less marked. The first menstrual blood contains a M.U. in 4 to 5 c.c. corresponding to 5 to 6 times the concentration of that found in the circulating blood.

The threshold mechanism which keeps the blood level low during the intermenstrual, and permits the sudden rise at the premenstrual time, has not been fully clarified.

Excretion through the kidneys and feces has been determined, as well as the excretion through the menstrual blood, but the governing mechanism has not been laid bare.

We have found 2 periods in the menstrual cycle during which there appears to be an increased excretion of female sex hormone in the urine which however never reaches above 1 to 2 M.U.L. This is in marked contrast to pregnancy conditions when from 5 to 10,000 M.U.L. may be excreted.

In pregnant women the hormone level is constantly high compared with non-pregnant females. In 30–40 c.c of blood a +3 or +4 is maintained from the 8th week on until the onset of labor. From the beginning of impregnation until the onset of labor, the anterior pituitary hormone may be demonstrated in the urine of pregnant women according to Zondek and Aschheim. With the expulsion of the placenta, an abrupt drop of the hormone level in both the circulating blood and urine of puerperal women takes place and this low level persists during lactation unless the menstrual cycle is re-established.

As disturbances in the sex sphere of women are due in many cases to factors outside of the genital tract, the methods of examining a female patient are detailed, attention being called to those congenital and acquired deviations which most frequently react upon the pelvic organs of the female. It has been found that most patients can be classified under 4 types, the normal feminine, the infantile, the eunuchoid, and the virile. In addition, more marked endocrine disturbances enable us to differentiate pituitary, thyroid, adrenal and gonadic groups.

In this study attention has been largely focused upon the female sex hormone test, but such useful aids as blood examinations, sugar tolerance, basal metabolism, X-ray of the sella turcica, examination of the visual fields, and study of the autonomic nervous system have not been neglected.

On the basis of these studies, the patients examined have been divided into various groups which include women suffering from hypofunction of the ovaries, hyperfunction of the ovaries, major endocrine disturbances. Furthermore, the test has been utilized in the diagnosis of pregnancy and in the differential diagnosis between pregnancy, amenorrheas, and non-pregnant enlargements of the uterus. The function of the ovaries left in after hysterectomy, the determination of sex in the presence of malformation have likewise been reviewed in the light of this test.

Hypofunction of the ovaries in the vast majority of cases documents itself in the form of amenorrhea. Amenorrheas may be divided into primary and secondary amenorrheas.

Puberty occurs in the absence of menstruation as does pregnancy. By means of the test it was found that among primary amenorrheas, patients with regular subthreshold cycles, patients with irregular cycles, and patients with entire absence of cycles can be distinguished. These differences in blood examination are of great prognostic importance.

Among the cyclical amenorrheas, it was found that the duration was of less importance than the presence or absence of cyclical blood variation as demonstrated by our test. Amenorrheas due to obesity formed a large and interesting group. Here also the blood test was of great importance in formulating a prognosis.

At least the theoretical possibility of an amenorrhea due to persistence of the corpus luteum in the absence of pregnancy must be taken into account. In every instance of amenorrhea, the final decision as to the gravity or transient nature of the condition will depend upon the blood hormone picture found.

In the sterility group (exclusive of mechanical obstruction and of endocrine disease) the only striking finding was the uniformly low premenstrual blood threshold level. Tentatively I suggest that this type of sterile woman has a low threshold, and in consequence of this the uterine mucosa does not attain the proper pregravid endometrial preparation necessary for successful nidation. Further study is necessary to confirm this hypothesis.

A study of the eunuchoid and infantile group shows greater and more persistent deviation in the genital tract than is noted in other groups of women.

Menorrhagia or metrorrhagia as the result of hypofunction is of rare occurrence.

Hyperfunction of the ovaries can often be diagnosticated by means of the high amount of female sex hormone found in the circulating blood. Excess may cause exaggeration of the normal premenstrual uterine and breast phenomena. Vasomotor symptoms, symptoms of general tension, convulsive seizures, and subcutaneous hemorrhages have been noted.

Precocious puberty is the most striking documentation of hyperfunction of the ovaries. This may be due to anatomically not demonstrable ovarian changes, to ovarian tumors, or to new growths of the pineal or adrenal glands.

Excessive menstruation occurs in the form of puberty bleeding, as the menorrhagia and metrorrhagia of maturity, as preclimacteric and rarely as climacteric bleeding.

The puberty group shows a persistent and increased hormone content in the menstrual blood shed. The vein blood frequently shows no demonstrable cycle. The endometrium regularly is of the cystic hyperplastic variety.

Our examination of the menorrhagia and metrorrhagia of the sexually mature has thrown no new light upon this subject.

A sub-group is formed by the menorrhagia and metrorrhagia secondarily due to endocrine disturbances. In most instances the excessive bleeding is found at the beginning of a serious endocrine disease, such as hyperthyroidism or acromegaly, soon to be followed by a period of persistent amenorrhea as the underlying disease progresses.

The menorrhagias, the bearers of fibromyomta and of fibrosis uteri, compose a very fertile group in whom both this fact as well as our blood test examination, confirm the view that the symptoms are due to ovarian hyperactivity.

Other less well-defined but interesting clinical sub-groupings, due to hyperactivity of the ovary, include the rare dysmenorrhea membranacia, the virilistic women with cyanotic genitals, oversexed females, and the very rare post climacteric functional bleedings due to spontaneous rejuvenescence.

The test has been found most useful in distinguishing between pregnancy and other obscure conditions, but only after the 8th week of gestation has passed. Differentiations between amenorrhea due to diseases or to pregnancy, between pregnancy and fibromyomata, as well as pregnancy versus the menopause have been made. The test is also of value in demonstrating the viability of the fetus or placenta, or both, in cases where death of fetus, or detachment of the placenta is suspected.

Before the 8th week of gestation the test is of no value. For this reason it is of little importance in helping to diagnosticate early extra-uterine pregnancy. Like every biological test, at least 5 per cent of error may be expected.

By means of the test it has been found that ovaries left *in situ*, after hysterectomy, continue to function and produce a blood cycle such as was present before the operation. A similar cyclical variation in the blood content of patients without vagina has enabled us to determine the feminine sex of these malformed individuals.

In severe endocrine disturbances, additional data were obtained by means of the blood test. These included the study of pituitary conditions and thyroid diseases as well as a comparatively common syndrome which includes obesity, virilism, and hirsutes. Considerable prognostic importance was found to attach in these conditions upon the presence or absence of cyclical blood changes.

All evidence favors the opinion that the basal metabolism is but slightly affected by gonadal activity. Castration may reduce the gaseous exchange to a moderate degree. Desiccated ovarian preparations uniformly appear lacking in all biological activity.

Assay and biological standardization of the water soluble commercial extracts now placed upon the market show a woeful lack of potency and rapid deterioration of the products. Unpleasant local reactions may arise at the site of injection. The prices of these pharmaceutical preparations are prohibitive. Consequently I warn against their general use until better products are at our disposal.

My own efforts at therapy have been largely indirect. The sole endocrine preparation that has proved itself of real value has been thyroid extract which, in the form of desiccated thyroid substance, is of use in patients with lowered basal metabolism or as an adjuvant to dietary measures in obese patients who tolerate this medication.

Further measures have been directed toward roborant treatment in the asthenic, underweight and weak. So-called "stimulation" of the ovaries by means of X-ray should be confined to patients with amenorrhea and sterility in whom a distinct sub-threshold blood cycle can be demonstrated.

In conditions of hyperfunction various measures have proved of value. Precocious puberty due to ovarian tumor necessitates removal of the growth. In puberty bleedings hygiene, transfusion for severe anemia, repeated curettages, and as a last resort, temporary X-ray castration may be used.

In the bleedings of mature women, particularly women with sufficiently large families, no hesitation need be felt, if medical measures and repeated curettages have failed, in inducing amenorrhea by means of X-ray castration.

Preclimacteric hemorrhages demand immediate diagnostic curettage in order to exclude malignancy. X-ray castration is often required.

If, as is to be hoped for, the future places a concentrated and stable female sex hormone in our hands, this powerful drug will enable us to produce transient stimulation of the uterus. Upon the withdrawal of the drug a single menstruation should occur, but it must not be forgotten that the ovaries are not stimulated by the female sex hormone. The possession of a potent aqueous corpus luteum extract may enable us to control too frequent and too prolonged menstruation. Again this effect may be expected to be transient. On the other hand, an active anterior lobe pituitary preparation should enable us, when it has been successfully elaborated, to actually stimulate the ovaries to increased function and activity.

Throughout this presentation I have refrained from giving any definition of what the female sex hormone is. Our present knowledge permits us of giving only a very imperfect definition.

The female sex hormone is of simple composition (C, H, O). Chemically it shows lack of affinities and activity; biologically on the contrary, its activity is exerted at minute concentration. The activity is almost exclusively limited to Müller's ducts and the mammary glands. The effect produced by the female sex hormone fosters, matures, and stimulates all feminine qualities and attributes, as well as the organs needed for the perpetuation of the species.

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PART II

INCLUDING NOS. 491A–528

Note.—For References 1 to 491 inclusive, See Part I, page 149.

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